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PROGRESS

CLINICAL ASPECTS OF SULFAPYRIDINE THERAPY*

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PNEUMONIA has long been recognized as one of the more important of the lethal afflictions of mankind.

From Hippocrates,[1] who wrote of the importance of the "concoction" of the sputum; and Aretaeus,[2] who began his discussion with these words: "Animals live by two principal things, food and breath (pneuma); of these by far the most important is the respiration, for if it be stopped, the man will not endure long but immediately dies"; on to Osler's classic description of this disease as "Captain of the Men of Death," pneumonia has attracted the attention of the physician and investigator throughout the ages.

But despite the work of innumerable investigators, pneumonia for centuries remained a disease before which the physician stood almost without resource; for progress in medicine, though steady and persistent, often seems intolerably slow.

Not until the advent of type-specific immune sera was there any marked advance in the therapy of pneumonia, and great though this discovery was, the victory was but partial. For, though it was learned that the race of pneumococci embraced 32 serologically separable types—perhaps more—for but relatively few of these did it seem possible to develop therapeutically effective antisera.

Attention was focused, therefore, upon developing a specific and effective chemotherapy; not necessarily what Ehrlich defined as a *"therapia sterilisans magna"* but some drug which per se, or through its derivatives, might offer definite and demonstrable hope for the consistently efficient management and control of pneumonia in all its varied bacteriologic entities. Thus, for many years, this drug or that, this compound or the other, flourished for a while and, under the acid test of time and trial, passed on to obscurity.

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Appreciation of great discoveries in medicine is more often gradual than dramatically sudden; indeed, they often pass relatively unnoticed until fortuitous circumstances bring them to light.

Thus it was with the "Quellung" reaction, described by Neufeld, which, though now an essential prerequisite to the intelligent treatment of pneumonia, lay dormant for a quarter of a century. Thus it was also with the discovery of sulfanilamide which, though elaborated in 1908, remained with all its remarkable qualities unheralded and unsung until comparatively recent times.

As its widening range of capabilities became more and more apparent, it was inevitable that attempts should be made to find in sulfanilamide, or in its derivatives, some drug which, effective against the pneumococcus, would also possess a chemotherapeutic index rendering it safe for clinical use.

It is needless to list the innumerable substances synthesized in these attempts. If the resultant compound proved effective against the pneumococcus in experimental infections, all too often the chemotherapeutic index—the margin between the toxic and the therapeutically efficient dose—was too small for safety.

It was not until the report of Whitby[3] in 1938—it seems difficult to appreciate that it was only two and a half years ago—of the apparent efficiency of 2-sulfanilylaminopyridine, a substance elaborated by Ewins and Phillips in England, that there seemed some promise that the long-sought goal had been reached.

Whitby first reported the effectiveness of this compound against the pneumococcus, but it was the report of Evans and Gaisford[4] in the same year which first showed its application to the treatment of lobar pneumonia. Since then innumerable reports concerned with it have been written, with the end not yet in sight.

Though efficient chemotherapy for pneumonia seems in large measure to have been at last achieved, oddly enough we are still largely at a loss for a satisfactory explanation for, or a definite understanding of, the mode of action of sulfapyridine upon the pneumococcus. That it has an action the clinical response following its administration in pneumonia proves beyond dispute. That this clinical effect is dependent upon some mechanism yet to be explained is equally clearly shown by various laboratory and experimental studies.

It is well recognized that bacterial infection is primarily dependent upon the concomitant influence of several factors, some of primary and essential, others of ancillary, importance.

In addition to the inherent and acquired resistance of the patient and his tissues, the virulence, aggressiveness, and toxicity of the invading bacteria must all be taken into account. Only when a suitable balance or imbalance between these varied factors is present does infection occur.

When chemotherapy is effective, it is logical to look for the reason in its effect upon bacteria, and it is ordinarily to be expected that that effect will be mainly, if not entirely, bactericidal.

But the action of sulfanilamide and its derivatives, sulfapyridine among them, upon the pneumococcus is not bactericidal but apparently merely bac-

teriostatic. For while the pneumococcus remains alive and propagates itself in the presence of sulfapyridine, there is a definite lag in its growth, which appears to be definitely inhibited, establishing the drug as bacteriostatic rather than bactericidal.

We do not know how this bacteriostatic effect comes about; for while there are many theories and hypotheses, all must be regarded as speculative and unproved. This is, perhaps, not altogether surprising in view of the fact that the pneumococcus itself still remains in many respects somewhat of a bacteriologic puzzle.

Perhaps the most important advance toward an understanding of the pathogenicity of the pneumococcus was the discovery that its most important constituent, and the one most largely concerned in its action upon the tissues, resides in its capsule, the "specific soluble carbohydrate substance," as it is now called. Unfortunately, this substance is soluble and passes into the blood stream. In many respects the severity of the pneumonic infection is rather directly proportionate to the concentration in, and distribution of, this substance by the circulation.

We are still uncertain of the relationship between this capsular polysaccharide and the invasiveness of a particular strain of pneumococci. We do not know whether the polysaccharide may properly be regarded as the pneumococcus toxin, or whether the pathogenic ability and toxicity of the pneumococcus resides solely in its capsular substance or in part—or at all—in its somatic protein substance. These are but a few of the problems to be solved.

Granting the bacteriostatic effect of sulfapyridine upon the pneumococcus, what, if any, is the relationship and the importance of this effect to the clinical results obtained?

Is the bacteriostatic effect the ultimate explanation? Or does the bacteriostasis simply allow time for mobilization of the defensive mechanism of the patient so that the neutralization of toxic substances and the elimination of the pneumococcus by phagocytosis, bacteriolysis, and the contributory effect of opsonins may be effectively exerted?

While any or all of these may take place, or may be in some measure determined by bacteriostasis and thus play some part in recovery, it is difficult to believe that this is the whole story.

In view of the importance of the specific capsular polysaccharide in determining the pathogenicity and toxicity of the pneumococcus, does any evidence suggest a relationship between the clinical effects of sulfapyridine and some possible effect upon the capsular substance, its elaboration, or its concentration in the blood stream? May sulfapyridine prevent or inhibit the formation of capsular substance or in any way immobilize it or render it innocuous or relatively so? Any such assumption must postulate an effect upon the pneumococcus capsule consequent upon exposure to sulfapyridine. Is there any evidence suggesting such a possibility? Here at last we find a question to which a conditioned and reserved answer may be made, based upon observations by various workers in connection with pneumococcus typing by the Neufeld method.

As everyone now knows, type specificity is demonstrable in the Neufeld method by the occurrence of the Quellung reaction evidenced by a marked swelling of the pneumococcus capsule when brought in contact with its homologous immune serum.

Failure to type the pneumococcus may be dependent upon technical errors when the method is applied by those unskilled in bacteriologic procedures; it may be influenced by dissociation of the pneumococcus; certain types if not thereby shown to be identical, certainly show cross reactions with typing sera; failure to secure type differentiation may suggest there are more than 32 types; there is also definite and cumulative evidence that pneumococcus typing in the presence of sulfapyridine is not infrequently difficult and unsatisfactory, if not impossible.

It must be admitted, however, that the relationship of sulfapyridine to anomalous results in pneumococcus typing is still *sub judice*. Bullowa, Osgood, Bukantz, and Brownlee,[5] from a study of pneumococcus-infected marrow cultures, report that while pneumococci exposed to sulfapyridine may become distorted, develop long chains, and stain irregularly, they suffer no loss of capsule or type specificity.

Now, as the Quellung reaction upon which pneumococcus typing depends arises from an effect upon the capsule, if this effect is interfered with in the presence of sulfapyridine, it seems logical to postulate some effect of the drug upon the capsule. If this be so, then various speculations arise as to some possible relation to the specific capsular substance.

But again we encounter a maze of speculative hypotheses. Does sulfapyridine affect the permeability of the capsule, and, if so, would this increase the vulnerability of the pneumococcus to antibodies such as opsonins and hence to phagocytosis? Is the effect something other than some change in permeability? Does some change occur in the reactive ability of the capsule, and, if so, what is its nature? Or is there some alteration in the elaboration, mobilization, or solubility of the specific capsular substance? To none of these queries can a satisfactory or definitive answer yet be given.

It is both logical and empirical to assume a definite relationship between the chemotherapeutic efficiency and the concentration of the drug in question. As this has been shown to be in a large measure true of sulfanilamide, it was logical to assume that it must also hold true for sulfapyridine, the effective concentration in the blood being generally regarded as between 4 and 10 mg. per 100 c.c., with the latter as the upper limit of safety. However, there are fairly definite indications that suggest a lack of definite correlation between the blood concentration of sulfapyridine and the clinical result consequent upon its use in pneumonia.

Illustrative of such reports is that by St. George, Kraetzer, and Magee[6] of a carefully controlled series of 50 unselected cases. Their tables show concentrations of sulfanilamide in the blood varying from 1.3 to 6.5 mg. per 100 c.c., the latter being the highest they obtained. But, despite this variation within fairly wide limits, there was no apparent correlation with the ultimate clinical result nor was the incidence of recovery in any way definitely proportionate to the blood concentration.

It is recognized that the blood concentration is affected by factors such as nausea and vomiting, influencing the rapidity and degree of absorption, and also perhaps by others not yet clearly understood that may affect the degree to which the drug is acetylated or conjugated. Nevertheless, this and other similar reports are significant, even though at first they seem merely to further becloud an already obscure picture.

The intelligent consideration of any therapeutic agent must embrace not only its therapeutic effects, but also its nonspecific and undesirable by-effects; in other words, its toxicity for the human being.

Here we enter upon somewhat less uncertain ground for, though we may not always possess a clear explanation for their occurrence, we have cumulative and increasing clinical evidence of definitely toxic and undesirable by-effects of sulfapyridine for which it is necessary to be on guard and which, in any degree, it is highly desirable to avoid.

Remembering that sulfapyridine is synthesized from aminopyridine, it is not altogether amazing that there is some reason to believe that certain individuals, fortunately seldom encountered, may possess an inherent sensitivity to the drug which renders them more vulnerable to undesirable toxic effects. Because sulfapyridine is regarded as of relatively low toxicity, it does not follow that it can be safely administered without regard to the various toxic mainfestations which may appear, sometimes insidiously but not infrequently with disturbing rapidity.

Cyanosis is a very common occurrence after the administration of sulfapyridine but, even though marked, is rather generally regarded as of minor significance and not per se indicating withdrawal of the drug. As might be expected, there is some uncertainty whether sulfapyridine cyanosis depends upon sulfhemoglobinemia or methemoglobinemia. Cyanosis is not infrequently a symptom in pneumonia and may be present before, and hence independent of, the administration of sulfapyridine. But since the cyanosis in pneumonia is directly related to the oxygen saturation of the arterial blood, any increase consequent upon the administration of sulfapyridine cannot always be regarded with entire equanimity and unconcern.

The recent observation by Doughty,[7] corroborating the report by McGinty, Lewis and Holtzclaw,[8] that the cyanosis and the usual concomitant headache, weakness, and nausea following the administration of sulfanilamide may be effectually relieved by the administration of 20 mg. of nicotinic acid three times daily, is of definite value and may furnish a clue to the mechanism of these complications.

As the oral administration of sulfapyridine is at times impractical or impossible, the recent report of Haviland and Blake[9] is of interest. These investigators have found that a 0.15 per cent solution of sulfapyridine in normal (0.85 per cent) saline, or a 0.2 per cent solution in 5 per cent glucose solution, or in equal parts of normal saline and 5 per cent glucose, may be given intravenously without undesirable effects as long as the solutions are not allowed to cool below room temperature. The solutions are made by boiling and are allowed to cool spontaneously to room temperature. Such solutions have been given intravenously, subcutaneously, and within the arachnoid space and pleural cavity.

The main objection the authors cite is that large amounts (up to one or two liters) must be administered to procure adequate concentration. As in the presence of loss of fluid, salt, and glucose because of inadequate intake in comatose patients, or through vomiting, these substances may thus be replaced, the objection is of minor importance.

That sulfapyridine per se may produce a febrile reaction has been shown by many observers. It is important to differentiate this so-called "drug fever" from febrile exacerbations consequent upon the development of various complications. This may not always be easy for, though Graham, Warner, Dauphinee and Dickson[10] report that drug fever is not accompanied by leucocytosis, this has not been the consistent experience of others.

In keeping with the observation that vomiting following the administration of sulfapyridine depends largely, if not entirely, upon central nervous system irritation, it has been recorded that various other central nervous system disturbances may occur. The more common of these are vertigo, headache, malaise, and mental depression. Occasionally, excitement of a degree severe enough to be classed as a psychosis occurs in approximately 4 per cent of cases. Fortunately these reactions are transient and disappear promptly on withdrawal of the drug.

Reference has already been made to the wide variation in the blood concentration and to the difficulty of maintaining the blood concentration at any set level with any degree of constancy. Many factors are probably concerned: variations in absorption, variations in the excretion rate, and variations in the degree and rapidity with which changes in the composition and structure of the drug occur after absorption, notably in the liver.

Just as bacteria entering the blood stream do not remain there as particulate entities continually traversing the circulatory paths but are rather rapidly removed through the interlocking activities of various mechanisms, so chemotherapeutic agents entering the blood stream are likewise subject to varying influences. Obviously, they are at once diluted by the blood volume; they may in part combine with its protein or other constituents, with their activities thereby decreased, if not entirely inhibited; and they are frequently altered by the detoxifying function of the liver.

It is known that compounds having a free para-amino group—of which sulfapyridine is one—undergo acetylation in the liver as a result of which the free amino group is blocked. Sulfapyridine may thus be rendered more or less inert after absorption. Fortunately, though this reaction occurs in the liver, evidence of tissue irritation as a by-product is exceedingly rare, so that toxic hepatitis and jaundice are very uncommon.

Like many other drugs sulfapyridine may produce a drug rash, the particular importance of which is that, as such patients appear to be definitely photosensitive, the severity of rash may be greatly intensified by exposure to light, particularly strong sunlight.

Since sulfapyridine in both its free and conjugated (acetylated) form is excreted almost entirely by the kidney, and since it may be present in the urine in concentrations much greater than its solubility can account for,[11, 12] the occurrence of renal complications would not be unexpected. Thus a temporary de-

crease in the urea clearance, regarded as a toxic manifestation, has been reported by MacLeod,[13] and hematuria, generally microscopic, though occasionally macroscopic, has been noted by many observers.

The incidence of hematuria is low—0.8 per cent in 2,300 cases, in one report. Since it shows evidence of renal irritation and damage, it indicates the necessity for frequent and periodic urinalyses during the course of sulfapyridine therapy. It is also well to maintain a high fluid intake to dilute the concentration of the drug in the kidney and lead to its rapid elimination. As sulfapyridine is more soluble in alkaline than in an acid solution, the proposal of Long and Wood[14] that the urine be kept alkaline with sodium bicarbonate is worthy of note, even though it is purely empirical and as yet of unproved efficacy.

Of greater interest and more importance are the reports—relatively few but increasing in number—indicating the possibility of calculus formation as an aftermath of sulfapyridine therapy. Calculus formation in animals—the calculi being composed chiefly of acetylsulfapyridine—has been demonstrated experimentally by Toomey,[15] Antopol and Robinson,[16] and by Gross and associates.[17-20]

It is not yet possible to determine accurately how frequently this complication occurs, but some evidence suggests that the incidence may be greater in children than in adults. That, rarely, grave and even fatal consequences may follow is shown by a fatality reported by Long and Wood,[14] and one by Tsao, McCracken, Chen, Kuo, and Dale[22] following renal blockage and uremia; in view of these fatalities the importance of routine urinalyses with attention to the presence of red blood cells and sulfapyridine crystals in the urinary sediment is obvious.

By far the most important and most dangerous complications of sulfapyridine therapy are those resulting from depression of the hematopoietic system and manifested by the occurrence of hemolytic anemia and agranulocytic neutropenia.

While both of these are important, the anemia—while not infrequent and tending to be progressive—is comparatively the less dangerous, since it is practically always controllable by transfusion and withdrawal of the drug. Although apparently essentially hemolytic in type, the anemia may vary in its manifestations, in some cases resembling a definite chloro-anemia; in other instances the reduction in the red blood cell count is disproportionate to the reduction in hemoglobin. Present opinion inclines to the belief that, where the need for continued administration is urgent, anemia of itself is not a mandatory indication for withdrawal of the drug unless the anemia reaches a degree dangerous per se, and particularly when it cannot be controlled by transfusion.

Agranulocytic neutropenia may take place early after the administration of only a small amount of sulfapyridine. Usually, once begun, it progresses rapidly. Its importance lies in the fact that marked leucopenia invites invasion of the tissues by opportunist bacteria, normally quiescent and particularly present in large numbers and varieties on mucous surfaces (mouth, rectum, vagina), leading to necrosing and extensive tissue damage and destruction.

It is well recognized that granulocytopenia in malignant degree is the aftermath and expression of profound depression of the bone marrow. But the

majority of reports suggest that this is most likely to occur when the dosage of sulfapyridine has been large and long continued. In common with other forms of granulocytopenia dependent upon chemical intoxication, as contrasted to the form originally described by Schultz as agranulocytic angina which is now believed to be a filtrable virus disease, that following sulfapyridine therapy is more intense, and more likely to be dangerous in the presence of an individual sensitization or personal idiosyncrasy to the benzol ring. It is interesting to note that there appears to be no evidence that an individual can be sensitized to sulfapyridine itself.[23] Here, again, the importance of routine blood counts is evident, particularly because bone marrow damage may be manifest only after the cessation of sulfapyridine therapy.[24-26]

While this survey has been by no means comprehensive, it may serve to indicate that sulfapyridine therapy is still largely in the stage of clinical experimentation and, therefore, still in a state of flux.

It is unfortunate, in several respects, that the clinical use has far outstripped the careful and controlled laboratory study of sulfanilamide and its derivatives. It has been haphazard and empirical, to some extent based upon "wishful thinking," and has too often failed to appreciate that it is in large measure not clinical therapy, but clinical experimentation. What is the moral?

The moral, as I see it, is that if we are perforce engaged in mass clinical experimentation, we dare not evade the responsibility of so carrying on our investigations as to make them informative and ultimately of value. This means that our use of sulfapyridine and similar compounds that will inevitably follow it should be so safeguarded as to lessen the possibility of undesirable aftermaths, and so managed and controlled as to lead to a better understanding of its action, its capabilities, and the true indications for its use.

We, therefore, list the following desirable, and in some respects essential, adjuncts to the use of sulfapyridine in pneumonia:

1. The sputum should be typed in *every* case *before* the administration of sulfapyridine. Although this drug is not type specific in its action, knowledge of the type of pneumonia may lead to an evaluation of the place of sulfapyridine in treatment; it will give significant information in relation to prognosis; and it will indicate those cases in which the patient may best be served by combined serum and chemotherapy.

2. A blood count should be recorded *before* chemotherapy is begun, and the blood count should be frequently repeated as long as the drug is administered. It may profitably be continued for some days thereafter. It should be done *before* the institution of drug therapy to distinguish between antecedent anemia, leucopenia, or anemia arising from the disease per se and anemia due to drug toxicity.

The blood count should be *repeated* regularly during treatment because both anemia and granulocytopenia may appear insidiously and develop rapidly and progressively. Ravid and Chesner[27] have recently reported death from hemolytic anemia and nephrotic uremia on the third day of treatment after the administration of only 8 Gm. of sulfapyridine. As shown by post mortem, the uremia was apparently the aftermath of tubular blockage resulting from hemo-

siderosis and the presence of hemoglobin casts. There was also very probably some degeneration of the renal tubular epithelium reflecting a direct nephrotoxic action of the drug.

The blood should be observed *after* the cessation of chemotherapy because bone marrow damage may appear after the drug has been discontinued.

3. For reasons already discussed in some detail, routine and regularly repeated urinalyses should be a sine qua non of sulfapyridine therapy.

4. It is of great value whenever possible to take a blood culture *before* the institution of sulfapyridine therapy. First, because cultures taken *after* administration of the drug may be unreliable; second, because the blood culture, when positive, may confirm the sputum typing; third, because of the prognostic importance of bacteriemia as an indication for energetic treatment, the combined use of serum and drug therapy, or perhaps for the use of sodium sulfapyridine intravenously. Bullowa and his associates,[28] in a series comprising 324 adults and 113 children, report that their lowest mortality rate in adults was observed in persons treated early (one to four days) with serum and sulfapyridine. The results in children were inconclusive.

5. While there is some uncertainty concerning the clinical importance of determinations of the blood concentration levels during the course of sulfapyridine therapy, whenever possible these should be done routinely at regular intervals to discover their ultimate clinical significance and value.

6. It is well to mix sulfapyridine therapy with a generous measure of the ingredient recommended by Whistler for use on the palette of the artist, namely, brains.

I am aware that in this somewhat discursive presentation, I have developed more problems and envisaged more difficulties than I have been able or, indeed, have attempted to solve. So much so that one might be tempted to echo the words of General Wolfe in one of his dispatches to Lord Pitt: "There is such a choice of difficulties that I find myself at a loss how to determine."

We may also remember the axiom of Oliver Wendell Holmes that, "Knowledge, like timber, should not be used until it is well seasoned."

If, in perspective, the vista seems one of doubt, of difficulty, even of confusion, we need not necessarily be unduly discouraged. Remember that the mills of science, like those of the gods, grind slowly—but they grind! And if, under the keen handling of the wise, the patient and the long enduring, their little grist grows even smaller as the chaff is winnowed away, that which is left is fine and pure and unalloyed, and from it comes strength and stimulus for the fight against disease.

I believe that we are only on the threshold of the successful chemotherapy of pneumonia. Sulfapyridine is but the forerunner of many sulfanilamide compounds to come. We have only to recall that it first became known as M & B 693, indicating that it was only one of many compounds synthesized in the laboratories of May and Baker at Dagenham, England, to realize the patient persistence of the investigator.

We have only begun the conquest of pneumonia, but we can now envision a conquest; heretofore we have had only a nebulous hope. We have taken the

first and the hardest step and may well expect that those to follow will be neither few nor laggard in their coming.

I hope this discussion will not have seemed entirely enveloped in uncertainty and disagreement. If this be so, let me recall the words of Herbert Spencer: "Mankind, in its progress, passes through three stages: the unanimity of the ignorant; the disagreement of the inquiring; and the unanimity of the wise."*

*ADDENDUM: Since this paper was written, two further communications have appeared concerning the mode of action of sulfanilamide.

Lockwood and Lynch[29] believe, from their investigations, that the bacteriostatic and, as they state, "very limited bactericidal action in vitro" of sulfanilamide are dependent upon the concentration of the drug and the concentration of "peptone" in the media. "Peptone," in this connection, they define as any product of protein digestion, whether artificially elaborated or arising through the operation of natural enzymatic processes.

They demonstrated experimentally in cultures that as little as 0.01 mg. of peptone per cubic centimeter definitely affects the bacterial population curve in that even these small traces had a definite inhibitory influence upon the action of sulfanilamide. They suggest as a tentative explanation of the action of sulfanilamide that the drug interferes with the ability of susceptible bacteria to utilize for food the protein-split products in serum, and that death thus occurs through starvation and autolysis. The inhibitory effect of peptone, they suggest, arises from the fact that it supplies an excess of easily assimilable nitrogenous material which enables the bacteria to overcome the bacteriostatic action of sulfanilamide.

Another recent contribution to the mechanism of sulfanilamide action is that of Green[30] based upon extensive experiments, mainly with *Br. abortus*. Green suggests that the action of sulfanilamide depends upon three factors:

(a) The rate of proliferation in the initial growth phase. If, in a fluid medium, the bacterial density reaches a certain level, which Green calls "critical," there is an accumulation in the medium of some antisulfanilamide factor sufficient to prevent further sulfanilamide effect. Possibly Green's "antisulfanilamide factor" may be the "peptone" of Lockwood and Lynch.

(b) The rate at which sulfanilamide reaches a concentration in the bacterial cell. This, he says, depends mainly upon the concentration of sulfanilamide in the medium, possibly in part on the nature of the bacterial envelope.

(c) The permeability of the capsule to the antisulfanilamide factor which is either released or formed during autolysis of the dead or dying bacterial cell and which diffuses out into the surrounding medium. He suggests that the rate of such release and diffusion may be related to the chemical nature of the capsule and/or cell membrane peculiar to the bacterial species.

Green calls his antisulfanilamide factor the "P" or proliferation factor and suggests that it stimulates the same enzyme reaction in the bacterial cell which sulfanilamide inhibits, the reaction being probably fundamental in the metabolism of most, if not all, bacteria, but not in that of other living cells. This "P" factor is not species specific in source or action. He believes it probable that the sensitivity to sulfanilamide of any bacterial strain is conditioned by the rate at which the "P" factor is liberated by the autolyzing organism and that, under certain conditions, a balance may exist between sulfanilamide and the "P" factor when the organism, though still living, cannot reproduce.

This hypothesis may explain the clinical observation that infections apparently under control after the administration of sulfanilamide are exacerbated when the administration of the drug is stopped too soon.

Also of clinical importance is Green's observation that sulfanilamide-resistant strains may develop and that, under certain conditions, sulfanilamide may even act as a stimulator of bacterial growth because of the elaboration of the "P" factor.

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CLINICAL AND EXPERIMENTAL

HYPERTENSION IN RELATION TO HEIGHT*

Its Variation With Body Build and Obesity

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NO RIGIDLY controlled statistical study on a large representative sample has ever established a correlation between height and blood pressure. This question is apparently settled in medical literature, since the majority of workers in the field have not been able to show any such relationship. Alvarez and Stanley,[1] most often quoted in this connection, say, "There is no correlation with height." Wunderlich,[2] in a study of German students, found "no correlation with stature." Reed and Love[3] on a large group of United States Army Officers modified the statement somewhat and said the blood pressure was "but slightly, if at all, correlated with height." Stocks and Karn[4] stressed the fact that even in early life, with other factors held constant, systolic pressure is not correlated with height.

Hunter[5] found a slight increase in pressure with height, as did the Joint Committee on Mortality.[6] This increase was greatest at an early age and least later in life. It must be remembered, however, that in many insurance studies height is obtained merely by questioning the prospective policy holder. Rucker,[7] in a review of the literature, stated that at a very early age, before puberty, blood pressure is usually said to be correlated to length of the body, but Richey[8] found that even this correlation disappeared when weight was held constant.

In contrast to these statistical studies the clinical impression among physicians is that there is a relation between hypertension and short stature. Fishberg,[9] and other writers, characterized the hypertensive person as *short* and stocky. The fact that many men say "short and stocky" implies a height relation. We, therefore, find that the statistical evidence shows no correlation between stature and blood pressure, while clinicians are impressed that hypertension is found more commonly among short persons.

In our study of 10,883 persons we found a positive correlation of height to blood pressure in the different build and weight groups, a finding contrary to the general clinical impression.

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STATISTICAL ANALYSIS

The distribution of 10,883 persons according to age, weight, sex, etc., details of examination, and careful estimation of blood pressure, build, and obesity are all presented in previous papers.[10, 11] In general, the persons were examined in the nude. Weight was recorded on a beam scale; maximum height was obtained to a tenth of an inch; chest circumference was measured with a steel tape slightly above the nipple line during quiet breathing. Blood pressure was recorded with a mercury manometer on the left arm of the seated subject. The group was representative of the adult population between the ages of 20 and 70* years.

DISTRIBUTION OF HEIGHT

The average height of the men was 68.3 inches (68.2 inches when standardized to the United States population of 1934), with a standard range of 65 to 71 inches (Table I). This average is only a few tenths of an inch lower than that found by most other studies on the distribution of height. The Medico-Actuarial Investigation[13] found an average height of 68.5 inches. The average height of the women was 63.6 inches, with a standard range from 61 to 66 inches. Other studies on women show substantially the same average height. Dublin[14] found 64.3 inches, while the Medico-Actuarial Mortality Investigation[13] found 64.2 inches.

TABLE I

HEIGHT DISTRIBUTION OF 7,478 MEN AND 3,405 WOMEN

	MEN	WOMEN
Mean height	68.3 ∓ 0.07	63.6 ∓ 0.06
Median height	68.4	63.6
Mode height	68.6	63.6
Standard deviation	2.8	2.5
Standard range	65-71	61-66
Percentage under 60 inches	–1	7
Percentage 60-65 inches	11	66
Percentage 65-70 inches	61	26
Percentage 70 inches and over	27	–1

TABLE II

MEAN HEIGHT IN INCHES OF 7,478 MEN AND 3,405 WOMEN IN EACH DECADE

AGE	20-29	30-39	40-49	50-59	60-69	70 AND OVER
Men	68.7	68.3	68.0	67.8	67.5	67.3
Women	63.9	63.7	63.4	63.3	62.6	61.6

With an increase in age there is a slight decrease in the average height of a large group of persons (Table II). The greatest average height in young men was 68.7 inches; at 40 years of age it decreased to 68.0 inches, and at 70 years it was 67.3 inches. The group of women showed the same tendency. At 20 years of age the mean height was 63.9 inches; at 40 years it was 63.4 inches, and over

*For detailed description see: Robinson, S. C., and Brucer, M.: Range of Normal Blood Pressure, Arch. Int. Med. 64: 409, 1939.

70 years of age it was 61.6 inches. In both men and women the median and modal heights follow a similar age curve. This diminution of height represents a physiologic process of shrinking that most persons experience due to compression of the intervertebral disks, and also to postural changes.

But much more interesting than this decrease of the average stature of man with years is the tendency for the number of tall men and women to decrease after the third decade. Whereas at the age of 20, 5.7 per cent of the men are over 72 inches in height, after 60 years less than 1 per cent of the men are very tall. While 4.6 per cent of women are taller than 67 inches in the third decade, less than 1 per cent of women over 60 years of age are very tall. This is a very striking and statistically significant finding. We do not have an adequate explanation for this phenomenon, but shall discuss it later in terms of hypertension in the tall person. Increased mortality among tall persons is one of the explanations that must be seriously considered. On the other hand, the age incidence of short men and women increases as the group gets older. There are comparatively more short men in the older age groups than in the younger age groups.

TABLE III

INCIDENCE OF TALL MEN AND WOMEN AT VARIOUS AGES

AGE	20-29	30-39	40-49	50-59	60-69	70 AND OVER
Percentage of men under 63 inches	1.1	3.1	4.1	3.7	4.1	5.5
Percentage of men over 70 inches	19.5	14.5	13.1	11.1	8.8	2.8
Percentage of men over 72 inches	5.7	3.7	3.5	2.3	0.9	0
Percentage of women under 59 inches	1.0	2.6	4.4	5.0	6.6	20.0
Percentage of women over 65 inches	19.7	19.2	14.6	15.5	5.7	0
Percentage of women over 67 inches	4.6	3.5	3.3	2.6	0.9	0

RELATION OF HEIGHT TO BLOOD PRESSURE

If the body builds and weights of individuals are mixed, as they are in the chance distribution of any sample, height shows a slight correlation to blood pressure* (Table IV). The mean blood pressure of the men shows the short groups to have slightly higher systolic and diastolic pressures than the tall groups (Fig. 1). The short women have higher mean systolic and diastolic pressures than the tall women.

In order to study the possible effect of height on blood pressure, we divided the group into three height classifications: the very short, the medium, and the

*The correlation coefficients for the relation of height and blood pressure are as follows:

Systolic Blood Pressure

Men	*Women*
r hs = −0.037 ∓ 0.012	r hs = −0.095 ∓ 0.017
η hs = 0.061	η hs = 0.127
ζ hs = 0.003 ∓ 0.003	ζ hs = 0.007 ∓ 0.0003

Diastolic Blood Pressure

Men	*Women*
r hs = −0.002 ∓ 0.012	r hs = −0.030 ∓ 0.017
η hs = 0.051	η hs = 0.072
ζ hs = 0.001 ∓ 0.0007	ζ hs = 0.004 ∓ 0.002

Thus each correlation is a linear negative one and so small that without further careful investigation it would seem that height is not at all significant as a correlation to blood pressure.

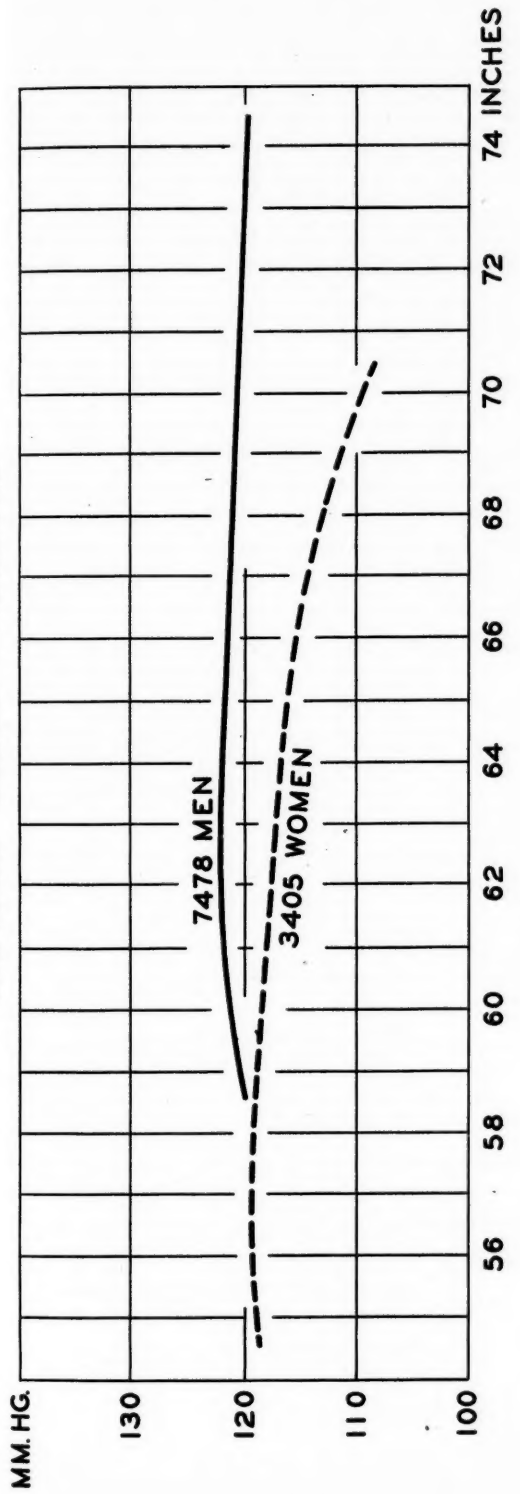

Fig. 1.—The influence of height on blood pressure. In the total group, with various builds and weights indiscriminately mixed, the average blood pressure is slightly higher in short men and women than in tall.

TABLE IV

RELATION OF HEIGHT TO BLOOD PRESSURE

HEIGHT IN INCHES	UNDER 63	63-64	65-66	67-68	69-70	71-72	73-74	75 AND OVER	ALL HEIGHTS	CORRELATION DATA		
Systolic, 7,478 Men											HEIGHT	BLOOD PRESSURE
No. of persons	237	709	1,541	2,099	1,814	799	231	48	7,478	Mean	68.2 ∓ 0.03	121.0 ∓ 0.20
										Mode	68.5	115.9
Mean pressure	120	123	122	121	120	120	120	120	121	Std. dev.	2.83	17.35
Median pressure	117	121	120	119	119	120	118	119	119	Skewness	-0.11	0.29
Modal pressure	112	115	117	115	117	119	113	119	116	Coef. var.	4.15	14.34
										Coef. corr.	$r = -0.037 \mp 0.012$	
% under 110 mm.	28	22	23	21	22	22	19	23	22	Corr. ratio	$\eta = 0.061$	
% 140 mm. and over	14	15	13	10	9	8	10	8	11	Test lin.	$\zeta = 0.003 \mp 0.0003$	
Diastolic, 7,478 Men												
No. of persons	237	709	1,541	2,099	1,814	799	231	48	7,478	Mean	68.2 ∓ 0.03	74.4 ∓ 0.12
										Mode	68.5	71.1
Mean pressure	73	75	75	74	74	74	75	74	74	Std. dev.	2.83	10.44
Median pressure	72	73	74	73	73	73	74	73	73	Skewness	-0.11	0.32
Modal pressure	71	70	71	71	72	71	72	71	71	Coef. var.	4.15	14.03
										Coef. corr.	$r = -0.002 \mp 0.012$	
% under 70 mm.	37	30	29	30	30	32	24	33	30	Corr. ratio	$\eta = 0.051$	
% 90 mm. and over	9	9	8	7	6	6	6	4	7	Test lin.	$\zeta = 0.001 \mp 0.0007$	

HEIGHT IN INCHES	UNDER 59	59-60	61-62	63-64	65-66	67-68	69-70	71 AND OVER	ALL HEIGHTS	CORRELATION DATA		
Systolic, 3,404 Women											HEIGHT	BLOOD PRESSURE
No. of persons	109	392	887	1,055	674	246	38	3	3,404	Mean	63.6 ∓ 0.04	117.0 ∓ 0.35
										Mode	63.6	112.4
Mean pressure	124	121	117	117	116	112	112	106	117	Std. dev.	2.45	20.22
Median pressure	114	115	113	114	113	112	109	105	113	Skewness	-	0.23
Modal pressure	112	103	107	107	107	112	112	103	112	Coef. var.	3.85	17.28
										Coef. corr.	$r = -0.095 \mp 0.017$	
% under 110 mm.	37	38	39	38	40	42	55	67	39	Corr. ratio	$\eta = 0.127$	
% 140 mm. and over	25	16	10	9	8	4	3	0	10	Test lin.	$\zeta = 0.007 \mp 0.003$	
Diastolic, 3,404 Women												
No. of persons	109	392	887	1,055	674	246	38	3	3,404	Mean	63.6 ∓ 0.04	71.0 ∓ 0.20
										Mode	63.6	70.1
Mean pressure	72	72	71	71	71	70	73	64	71	Std. dev.	2.45	11.58
Median pressure	71	71	71	71	71	71	71	65	71	Skewness	-	0.08
Modal pressure	69	69	70	71	70	72	69	-	70	Coef. var.	3.85	16.31
										Coef. corr.	$r = -0.030 \mp 0.017$	
% under 70 mm.	49	45	46	46	47	47	45	67	47	Corr. ratio	$\eta = 0.072$	
% 90 mm. and over	7	9	6	5	7	4	8	0	6	Test lin.	$\zeta = 0.004 \mp 0.002$	

very tall.* Because this division is used only for comparisons between the extremes, the arbitrary nature of the division makes little difference. If the divisions had been made either an inch higher or an inch lower, the results would have been essentially the same. It would be well, however, to establish a standard division in future statistical work.

TABLE V

MEAN SYSTOLIC PRESSURE IN VARIOUS HEIGHT CLASSIFICATIONS

HEIGHT	7,478 MEN	3,405 WOMEN
Short	121.9 ∓ 0.90	117.6 ∓ 0.82
Medium	120.0 ∓ 0.49	115.1 ∓ 0.70
Tall	119.7 ∓ 0.56	114.1 ∓ 1.01

TABLE VI

MEAN DIASTOLIC PRESSURE IN VARIOUS HEIGHT CLASSIFICATIONS

HEIGHT	7,478 MEN	3,405 WOMEN
Short	75.3 ∓ 0.51	70.6 ∓ 0.39
Medium	74.4 ∓ 0.29	70.1 ∓ 0.41
Tall	73.7 ∓ 0.34	70.1 ∓ 0.66

The blood pressure in these three height groups shows an interesting comparison (Table V). There is a 2.2 mm. difference in systolic pressure between short and tall men and 3.5 between short and tall women. These differences are statistically significant.† It is improbable that a purely chance distribution would cause such a difference.

The situation in diastolic pressure is similar to that in systolic pressure (Table VI). The difference of 1.6 mm. between the short and tall men is significant of an actual difference not due to chance errors in distribution.‡ The

*Because the differences between short and tall individuals were unexpectedly worthy of consideration, the two extreme height groups were augmented by a random selection from our files of 915 additional tall and short individuals.

Men

359 (plus 157) short = under 66 inches
1,212 medium = 66 to 70 inches
290 (plus 534) tall = 71 inches and over

Women

734 (plus 208) short = under 63 inches
767 medium = 63 to 65 inches
296 (plus 16) tall = 66 inches and over

These augmented groups were not used in Tables I and II, but were used only for the blood pressure comparison between short and tall persons so as not to affect the random character of the total sample. All discussions concerning age, height, and obesity (ponderal index) distributions are based on figures from the original sample of 7,478 men and 3,405 women. The discussion of build and blood pressure is based on a portion of this sample, 1,861 men and 1,797 women. The blood pressures of the original and the augmented groups are practically identical, which supports the genuine character of the results.

Men			*Women*		
Short	=	65 inches and under	Short	=	62 inches and under
Medium	=	66 to 70 inches	Medium	=	63 to 65 inches
Tall	=	71 inches and over	Tall	=	66 inches and over

†The standard difference between the pressures of the short and tall men shows that to be significant the difference must be at least 2.16 mm. Between short and tall women it must be at least 2.6 mm. Because the actual differences are slightly greater than this standard difference, it is improbable that the difference is due to a pure chance distortion of the distribution.

‡The standard difference shows that to be significant the difference between the diastolic pressure of short and tall men must be at least 1.22 mm. For women the difference in diastolic pressure must be at least 1.52 mm.

difference of 0.5 mm. for women is not significant. Thus, in our total amalgam groups short men have slightly higher average systolic and diastolic pressures than tall men, and short women have slightly higher average systolic pressure than tall women.

The incidence of low and high pressures in short and tall men and women brings out more significantly the extent of these differences in the two extreme height groups. Twenty-five per cent of the short men have low systolic blood pressures, and 22 per cent of the tall men have systolic pressures under 110 mm. Forty-one per cent of short and tall women have systolic pressures under 110 mm. On the other hand, both men and women show a higher incidence of high pressures in the short groups than in the tall. Fourteen per cent of short men have high systolic pressures of 140 mm. and over, while only 9 per cent of tall men show these same high pressures. Twelve per cent of short women have a high systolic pressure, while only 5 per cent of tall women have pressures of 140 mm. and over.

Diastolic pressure shows a somewhat similar incidence variation. Again there is not much difference between the incidence of low pressures; 27 per cent of short men have diastolic pressures under 70 mm., while 29 per cent of tall men have these same low pressures. Fifty-one per cent of short women have low diastolic pressures, while 47 per cent of tall women have diastolic pressures under 70 mm. The incidence of high diastolic pressures shows significant differences between short and tall persons. Short men show an incidence of 8 per cent diastolic pressures of 90 mm. and over, while tall men show considerably fewer, only 5 per cent, high diastolic pressures. Short women show an incidence of 8 per cent and tall women 5 per cent high diastolic pressures.

It is thus seen that the mean as well as the distribution of low and high pressures both prove that the short men or women have more hypertension than their tall brothers or sisters. Offhand, without any further statistical breakdowns of the height groups studied, one might be led to believe, first, that stature per se exerted a strong influence on blood pressure and, second, that the short person was the one influenced more unfavorably. When the build and weight of each height group are held constant, it will be shown that the former statement is correct and the latter is false.

The data just presented were of the totals of each height group. To arrive at a true correlation of height to blood pressure it is necessary to separate our groups according to age, weight, build, and other factors that are known to be related to blood pressure.[10-12] If the short group has a larger number of persons that are fat and stocky, and if obesity and stockiness are related to hypertension, then the short group will show more hypertension but it will not necessarily be due to stature. To avoid such an error we must correct for these disturbing factors. This we do by holding the build and the weight factors constant in the different height groups.

RELATIONSHIP OF HEIGHT TO BLOOD PRESSURE IN SPECIFIC BUILD GROUPS

It is generally recognized by most anthropometrists that the gross morphology of mankind can be divided roughly into two contrasting build types,

the thin and the broad. The frame of the broad build person can be differentiated from the very narrow type of build by the chest/height ratio. This figure is obtained by dividing the chest circumference in inches by the height in inches. It is to be noted that the measure of weight is not used at all to arrive at this ratio. The simplicity of the index is its main asset and can be pictured easily by the rule-of-thumb statement that the chest circumference should be one-half the height, or a chest/height ratio of 0.50.

When the group is broken down into linear, intermediate, and lateral build classifications, slight differences are apparent (Table VII). Neither the linear nor the lateral build men show a statistically significant difference in mean or modal systolic or diastolic pressure between the short and tall classifications.

TABLE VII

COMPARISON OF MEAN AND MODAL PRESSURES OF 2,552 MEN AT VARIOUS HEIGHTS IN SPECIFIC BUILD GROUPS

PRESSURE			LINEAR BUILD (SLENDER)	INTERMEDIATE BUILD	LATERAL BUILD (BROAD)
Systolic	Short men	Mean	113.8	120.7	127.5
		Mode	107.5	112.7*	122.7*
	Medium men	Mean	113.7	120.5	125.3
		Mode	112.1*	110.3	119.0
	Tall men	Mean	116.8	120.8	130.6
		Mode	110.8	113.9	121.3*
Diastolic	Short men	Mean	70.5	74.2	79.6
		Mode	70.8	70.6	80.5
	Medium men	Mean	70.4	74.8	77.8
		Mode	72.5	70.6	81.1
	Tall men	Mean	71.1	74.9	81.5
		Mode	72.3	70.7	80.0

*Estimated mode.

TABLE VIII

COMPARISON OF MEAN AND MODAL PRESSURES OF 2,021 WOMEN AT VARIOUS HEIGHTS IN SPECIFIC BUILD GROUPS

PRESSURE			LINEAR BUILD (SLENDER)	INTERMEDIATE BUILD	LATERAL BUILD (BROAD)
Systolic	Short women	Mean	109.6	115.4	127.9
		Mode	107.2	105.2	113.0*
	Medium women	Mean	110.1	116.0	128.4
		Mode	106.5	112.1*	116.4
	Tall women	Mean	109.1	118.1	134.0
		Mode	109.7	112.7*	122.0
Diastolic	Short women	Mean	66.8	69.7	75.9
		Mode	62.3	70.0	71.1
	Medium women	Mean	66.9	70.8	77.0
		Mode	63.6	71.4	70.4
	Tall women	Mean	66.4	72.6	84.0
		Mode	63.1	71.1	79.5

*Estimated mode.

The short linear women show the same mean and modal pressures that are found in the tall linear women. However, the intermediate build women show slightly higher pressures if they are tall than if they are short (Table VIII). This difference is most marked among the women of lateral build. But the maximum difference of about 8 mm. between short and tall in the lateral build is not statistically significant. Notice that the small differences which

do appear, while not statistically significant, are reversed from what was found in the total group. Tall persons show a greater hazard of hypertension than short persons in specific build groups.

Had we stopped at this point in our statistical survey of the relation of height to blood pressure even in the build breakdowns, we could not show any statistical correlation. Other studies, thus far, have relied solely upon the mean and mode, and hence, were unable to demonstrate any correlation.

More important than the mean or modal pressure is a study of the percentage distribution of low and high pressures in each build group of short and tall men and women. The tall linear men show fewer low pressures and more high pressures than short linear men (Table IX).

TABLE IX

COMPARISON OF THE INCIDENCE OF LOW AND HIGH SYSTOLIC PRESSURES IN 516 SHORT AND 824 TALL MEN

PRESSURE		LINEAR BUILD % (SLENDER)	INTERMEDIATE BUILD %	LATERAL BUILD % (BROAD)
Low	Short men	47*	26	16
	Tall men	30	19	13
High	Short men	3*	14	18
	Tall men	5	10	29

*Small distribution—less than 50 short linear men.

TABLE X

COMPARISON OF THE INCIDENCE OF LOW AND HIGH DIASTOLIC PRESSURES IN 516 SHORT AND 824 TALL MEN

PRESSURE		LINEAR BUILD % (SLENDER)	INTERMEDIATE BUILD %	LATERAL BUILD % (BROAD)
Low	Short men	47*	30	16
	Tall men	39	24	13
High	Short men	3*	6	16
	Tall men	1	6	23

*Small distribution—less than 50 short linear men.

The same relation is apparent in diastolic pressure (Table X). Although there is a smaller percentage of high pressure among tall linear men than among short linear men, the distribution in this group is small.

More important are the comparative incidences among lateral or broad build men. Tall men show a greater incidence of hypertension, both systolic and diastolic, than short men, and less low pressure. Whereas 16 per cent of short lateral men have low systolic and diastolic pressures, only 13 per cent of tall lateral men have these same low pressures. Only 18 per cent of short lateral men have systolic hypertension, whereas 29 per cent of tall lateral men have high systolic pressures. Sixteen per cent of short lateral men have diastolic hypertension compared with 23 per cent high pressures among tall lateral men.

The women show a similar relation within their height classifications (Table XI). Tall linear women show less low pressure and more high pressure, both systolic and diastolic, than short women. Among the lateral build women this is more marked. Only 12 per cent of tall lateral women have low systolic

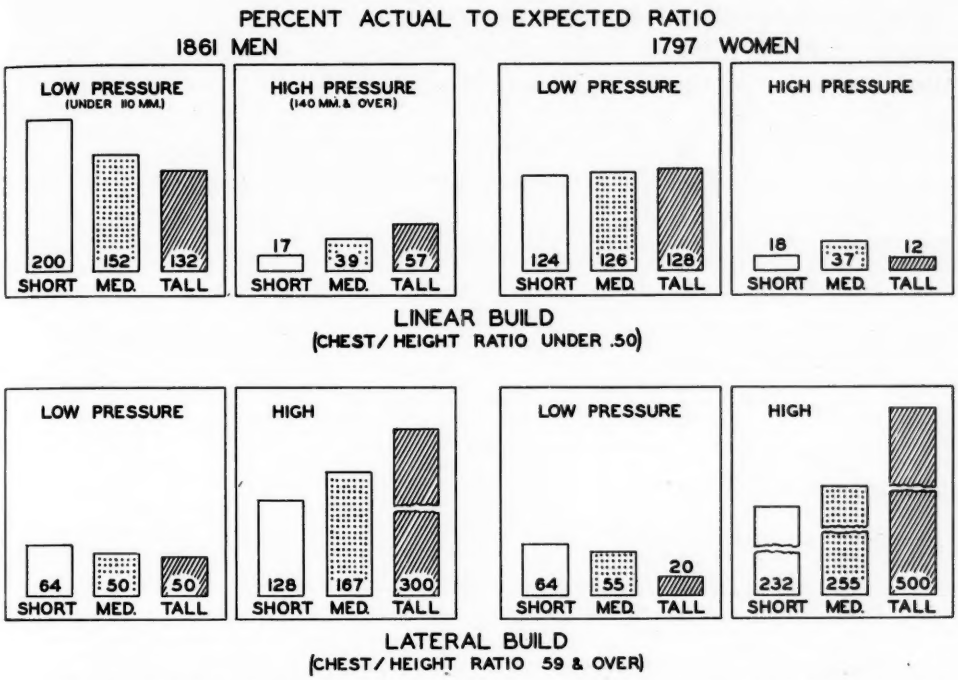

Fig. 2.—The influence of height on systolic blood pressure in specific build groups. The actual to expected ratio shows that in either linear or lateral build groups the tall men will show fewer low systolic pressures and more high systolic pressures than the short men. The same is true for lateral build, though not for linear build, women.

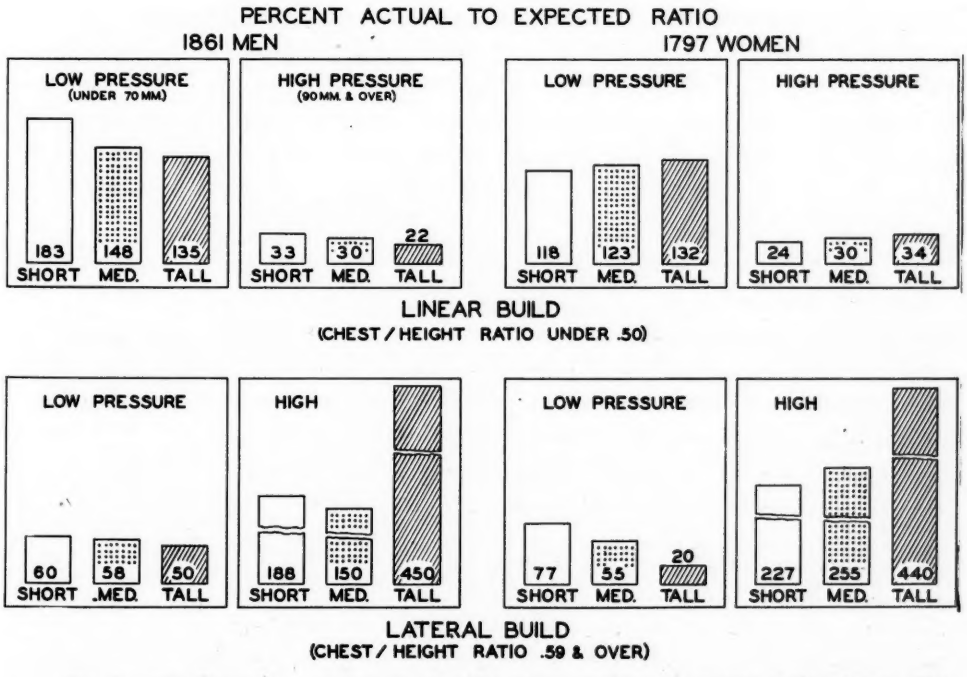

Fig. 3.—The influence of height on diastolic blood pressure in specific build groups. The actual to expected ratio shows that in the lateral or broad build group tall men and women will show fewer low diastolic pressures and more high diastolic pressures than the short men and women.

pressures, while fully 26 per cent of short lateral women have these same low pressures, under 110 mm. Only 12 per cent of tall lateral women have low diastolic pressures, while 36 per cent of short lateral women have these same low pressures (Table XII).

TABLE XI

COMPARISON OF THE INCIDENCE OF LOW AND HIGH SYSTOLIC PRESSURES IN 942 SHORT AND 312 TALL WOMEN

PRESSURE		LINEAR BUILD % (SLENDER)	INTERMEDIATE BUILD %	LATERAL BUILD % (BROAD)
Low	Short women	53	44	26
	Tall women	52	32	12
High	Short women	3	9	29
	Tall women	0.6	8	30

TABLE XII

COMPARISON OF THE INCIDENCE OF LOW AND HIGH DIASTOLIC PRESSURES IN 942 SHORT AND 312 TALL WOMEN

PRESSURE		LINEAR BUILD % (SLENDER)	INTERMEDIATE BUILD %	LATERAL BUILD % (BROAD)
Low	Short women	60	51	36
	Tall women	62	35	12
High	Short women	2	6	18
	Tall women	2	8	24

The lateral build women show the same trend as men in respect to high pressure, although the difference in systolic pressure between short and tall women is not significant. However, 24 per cent of tall women have diastolic hypertension as compared with 18 per cent of short women.

It is thus seen that the study of the incidence of low and high pressures in different build groups shows a significant difference between tall and short persons. Generally speaking, the tall individual has a lower incidence of low pressure and a greater incidence of high pressure. This is most marked in the lateral build groups.

The actual to expected ratio* brings out comparative differences in a more illustrative manner and corrects for changes in distribution between the various heights. First, we shall discuss the linear or thin build. By segregating these persons in each height group and then comparing the actual to expected ratio of low and high systolic pressures, we find that the short linear or thin men have one and one-half times as many low systolic pressures, and nearly that many more low diastolic pressures as the tall thin men (Tables XIII and XIV). However, in the case of high systolic pressures the short thin men

*The per cent actual to expected ratio is very simply calculated and is of use in illustrating the relative effect of the extremes of one array upon the dispersion of another correlated array. The technique is used here to show the unusual influence of a broad build on blood pressure. For example, if in any random group of 100 males, 24 are of the linear type and 13 are of the lateral type, and if there is no correlation of build to blood pressure, then the same ratio of 24 linear types to 13 lateral types should hold true in any group of individuals whether they have low, medium, or high pressures. This, however, is not the case. Among a group of 100 hypertensive persons there are actually only 9 linear types to 23 lateral types. This gives a per cent actual to expected incidence of 9/24, or a ratio of only 38 per cent of expected linear build hypertensive individuals as compared with 23/13, or a ratio of 177 per cent of expected lateral build hypertensive individuals. Simply stated, this means that among hypertensive persons the lateral build predominates in a ratio of over 4 to 1. When this is done in each height group, the ratios can be compared as they correct for changes in the height-build distribution.

have only one-third the ratio shown for tall thin men (Figs. 2 and 3). The difference is reversed for diastolic pressure. In comparison of the short and tall men of the lateral or broad build groups we also find that the short men have more favorable pressures. There are about two and one-half times as many systolic and diastolic hypertensives among the tall men as among the short lateral men. The ratio for low pressures is also slightly in favor of the short men.

TABLE XIII

PER CENT ACTUAL TO EXPECTED INCIDENCE OF LOW AND HIGH SYSTOLIC PRESSURES AT VARIOUS HEIGHTS IN LINEAR AND LATERAL BUILD MEN AND WOMEN

	LOW PRESSURE*	HIGH PRESSURE		LOW PRESSURE	HIGH PRESSURE
Linear Build* (Slender)					
Short men	200	17	Short women	124	18
Medium Men	152	39	Medium women	126	37
Tall men	132	57	Tall women	128	12
Lateral Build (Broad)					
Short men	64	128	Short women	64	232
Medium men	50	167	Medium women	55	255
Tall men	50	300	Tall women	20	500

*For the purpose of this and other similar charts the terms used are defined as follows:
Low pressure = Under 110 mm. systolic pressure and 70 mm. diastolic pressure.
High pressure = 140 mm. and over systolic pressure and 90 mm. and over diastolic pressure.
Linear or slender build = Chest / height ratio under 0.50.
Lateral or broad build = Chest / height ratio 0.59 and over.

This division is purely arbitrary and is used only to bring out the difference in the extremes of the distribution. Throughout this study finer subdivisions of chest/height ratio are usually given which merge from one build type into the other. It does not imply that the intermediate group is normal. The demarcation of normal levels is discussed in another publication.

TABLE XIV

PERCENTAGE ACTUAL TO EXPECTED INCIDENCE OF LOW AND HIGH DIASTOLIC PRESSURES AT VARIOUS HEIGHTS IN LINEAR AND LATERAL BUILD MEN AND WOMEN

	LOW PRESSURE	HIGH PRESSURE		LOW PRESSURE	HIGH PRESSURE
Linear Build (Slender)					
Short men	183	33	Short women	118	24
Medium men	148	30	Medium women	123	30
Tall men	135	22	Tall women	132	34
Lateral Build (Broad)					
Short men	60	188	Short women	77	227
Medium men	58	150	Medium women	55	255
Tall men	50	450	Tall women	20	440

This same type of relationship holds for women in the lateral group but not for those in the linear group (Tables XIII and XIV). There is practically no difference in the actual to expected ratio of low and high pressures between the short thin women and the tall thin women. But there are twice as many systolic and diastolic hypertensive persons in the tall lateral as there are in short lateral women and less than one-third the low systolic and diastolic pressures.

In both linear and lateral build groups tall men have a far stronger tendency to develop hypertension and less tendency to develop low pressure

than their shorter brothers. The same holds true for women of the lateral build. This same tendency is reflected in the actual to expected ratio of low to high pressures within a specific build-height group. There will be a ratio of 12 low pressures to 1 high systolic pressure among short linear men, almost 4 low to 1 high among medium height linear men, and only a little more than 2 low to 1 high systolic pressure among tall linear men. This does not hold for diastolic pressure among linear men or for systolic or diastolic pressure among thin women. However, in the lateral build group, an unusual difference was found in both systolic and diastolic pressures among both men and women. Among lateral build persons the ratio is reversed. The lateral build person of any stature is always more susceptible to high pressure than to low pressure, but the tall lateral person carries the greatest hazard of all. Among short lateral men there will be only 2 high systolic pressures to 1 low systolic pressure. Medium height men show a ratio of more than 3 high systolic pressures to 1 low systolic pressure, while tall lateral men show a ratio of 6 high systolic pressures to 1 low systolic pressure. The women show a similar pattern. Short lateral women have almost 4 high systolic pressures to 1 low systolic pressure; medium height women show a ratio of almost 5 high systolic pressures to 1 low systolic pressure; and tall women show a ratio of 25 to 1. In other words, high pressure is far more common among tall lateral persons than among short lateral persons.

Diastolic pressure in lateral build shows the same trend. Among short lateral men there will be a ratio of 3 to 1, and among tall lateral men there will be a 5 to 1 chance of having a high diastolic pressure. Short lateral women show only a 3 to 1 ratio of high to low diastolic pressures, and tall lateral women show 22 high pressures to 1 low pressure. This extreme difference in the tall systolic and diastolic is erratic because of the small number of persons.

Thus it is apparent that for both men and women the tall lateral or broad person showed a greater predilection for systolic and diastolic hypertension than the short lateral person, and a lesser tendency to low pressure.

SUMMARY OF RELATION OF HEIGHT TO BLOOD PRESSURE IN SPECIFIC BUILD GROUPS

The summary of the relation of height to blood pressure in specific builds is brought out in the explanation of an apparent contradiction. We have shown that in our total group of 10,883 men and women the short persons had a higher mean pressure than the tall. On the other hand, after a study of the incidence of low and high pressures and the actual to expected ratio was completed in different build groups, we found that the tall individual was more susceptible to hypertension, especially if he was of the lateral build type. The increase in blood pressure of the tall person over the short *lateral* person is much greater than the increase of the mean pressure of the short person over the tall person in the total group. This is apparently a contradiction and must be explained. The explanation is to be found in the comparative distribution of build types in short and tall persons. In another paper it was shown that any lateral build person is more susceptible to hypertension than the linear type. Roughly, about three times as many lateral persons as linear persons are hypertensive. It can be seen that if there are more lateral builds among short men we would expect more

hypertension in the short group. This is precisely what we found. Twenty-four per cent of the short men are of the lateral build, while only 5 per cent of the tall men have a lateral build. The same is true for women. Thus in any sample of the population there will be far more short persons than tall lateral persons (Fig. 4). Although the tall lateral person is more susceptible to hypertension than the short lateral person, this increased susceptibility is more than offset by the greater number of short lateral persons in the total group. Hence, all short men together show a higher mean pressure than all tall men.

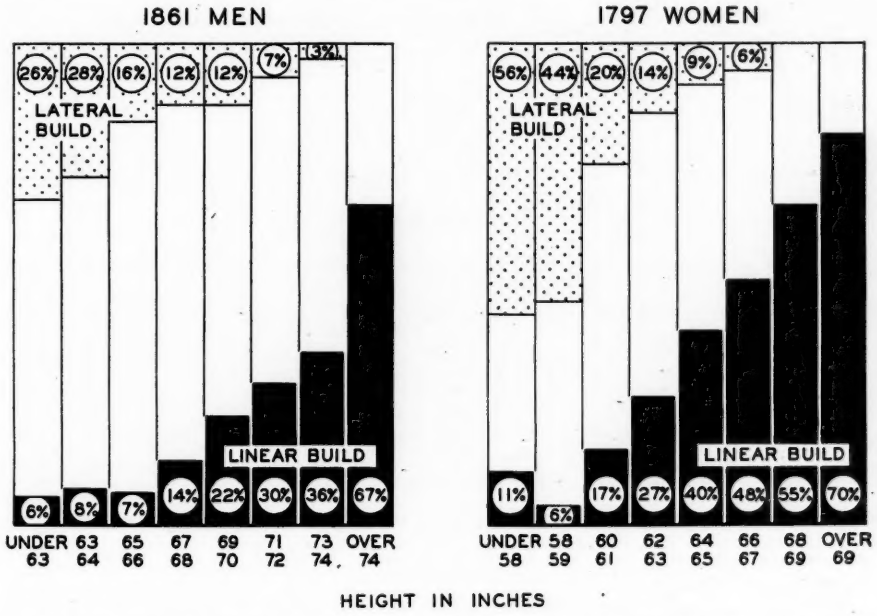

Fig. 4.—The distribution of linear and lateral build at different heights. Lateral or broad build is most frequently found in short men and women, and linear or thin build is most frequent among tall men and women. Blank sections indicate the intermediate build.

The clinical impression that the hypertensive individual is a short stocky man is therefore correct. The physician will see a far larger number of short stocky hypertensive persons and very few tall broad hypertensive ones merely because of the natural distribution of the lateral build in these two height groups. And yet the careful investigator must be aware that the tall broad individual is more susceptible to hypertension than any other person.

RELATION OF HEIGHT TO BLOOD PRESSURE IN SPECIFIC WEIGHT GROUPS

When the short, medium, and tall men and women are divided according to body weight groups, certain variations are important. The weight breakdown is done with the aid of the ponderal index, an index of relative obesity, the weight divided by the height. A low index (under 2.0) denotes a lightweight person, and a high index (2.5 and over) denotes an obese person. In a large sample of persons obesity is positively correlated to an increased blood pressure.

When the mean and modal pressures are compared in the various height-weight groups, no height difference in blood pressure is noticed in either light- or middleweight men (Table XV).

TABLE XV

COMPARISON OF MEAN AND MODAL PRESSURES OF 7,478 MEN AT VARIOUS HEIGHTS IN SPECIFIC PONDERAL INDEX GROUPS

PRESSURE			LIGHT-WEIGHT	MIDDLE-WEIGHT	HEAVY-WEIGHT
Systolic	Short men	Mean	117.6	121.5	128.5
		Mode	112.1	113.4	122.6
	Medium men	Mean	115.9	120.4	124.9
		Mode	110.5	114.4	118.0
	Tall men	Mean	117.5	118.3	123.9
		Mode	115.7	114.7	118.2
Diastolic	Short men	Mean	70.9	74.0	79.2
		Mode	71.2	70.7	78.9
	Medium men	Mean	70.5	73.6	78.5
		Mode	72.6	71.2	76.7
	Tall men	Mean	71.9	72.9	77.8
		Mode	70.7	72.0	73.3

TABLE XVI

COMPARISON OF MEAN AND MODAL PRESSURES OF 3,405 WOMEN AT VARIOUS HEIGHTS IN SPECIFIC PONDERAL INDEX GROUPS

PRESSURE			LIGHT-WEIGHT	MIDDLE-WEIGHT	HEAVY-WEIGHT
Systolic	Short women	Mean	111.8	119.8	127.6
		Mode	108.2	106.3	112.9
	Medium women	Mean	111.5	116.8	125.0
		Mode	108.2	112.1	110.9
	Tall women	Mean	109.8	115.6	121.2
		Mode	108.3	110.2	118.2
Diastolic	Short women	Mean	67.5	72.3	76.6
		Mode	63.3	71.1	73.3
	Medium women	Mean	67.6	71.5	75.7
		Mode	63.7	70.6	72.4
	Tall women	Mean	67.2	70.8	75.4
		Mode	67.5	70.5	70.3

TABLE XVII

COMPARISON OF INCIDENCE OF LOW AND HIGH SYSTOLIC PRESSURES IN 1,540 SHORT AND 1,078 TALL MEN (PONDERAL INDEX GROUPS)

PRESSURE		LIGHTWEIGHT PER CENT	MIDDLEWEIGHT PER CENT	HEAVYWEIGHT PER CENT
Low	Short men	35	23	16
	Tall men	24	24	16
High	Short men	9	13	22
	Tall men	3	7	14

TABLE XVIII

COMPARISON OF INCIDENCE OF LOW AND HIGH DIASTOLIC PRESSURES IN 1,540 SHORT AND 1,078 TALL MEN (PONDERAL INDEX GROUPS)

PRESSURE		LIGHTWEIGHT PER CENT	MIDDLEWEIGHT PER CENT	HEAVYWEIGHT PER CENT
Low	Short men	44	31	18
	Tall men	42	34	21
High	Short men	5	7	17
	Tall men	3	3	12

However, in the heavyweight men the tall group shows slightly lower systolic and diastolic pressures. At any height the men show higher pressures as they become heavier. However, the greatest increase is found in the short men where there is an increase of 10.9 mm. in mean pressure between lightweight and heavyweight men. Among tall men there is only a 6.4 mm. difference.

Lightweight, middleweight, and heavyweight women show the same systolic pressures at any height, but the short heavyweight women show a slightly greater diastolic pressure than the tall heavyweight women (Table XVI).

More informative than the mean and mode is a study of the distribution of low and high pressures among tall and short men in specific weight groups. Short men of any weight group show a greater incidence of high systolic and diastolic pressures than tall men (Tables XVII and XVIII).

A similar distribution is noted among women. The short women always show far more high systolic and diastolic pressures at any weight than tall women (Tables XIX and XX).

TABLE XIX

COMPARISON OF INCIDENCE OF LOW AND HIGH SYSTOLIC PRESSURES IN 1,383 SHORT AND 588 TALL WOMEN (PONDERAL INDEX GROUPS)

PRESSURE		LIGHTWEIGHT PER CENT	MIDDLEWEIGHT PER CENT	HEAVYWEIGHT PER CENT
Low	Short women	48	37	23
	Tall women	52	37	27
High	Short women	4	15	26
	Tall women	1	6	9

TABLE XX

COMPARISON OF INCIDENCE OF LOW AND HIGH DIASTOLIC PRESSURES IN 1,383 SHORT AND 588 TALL WOMEN (PONDERAL INDEX GROUPS)

PRESSURE		LIGHTWEIGHT PER CENT	MIDDLEWEIGHT PER CENT	HEAVYWEIGHT PER CENT
Low	Short women	59	40	30
	Tall women	56	47	28
High	Short women	3	7	15
	Tall women	1	5	10

The actual to expected ratio in the weight breakdown is not helpful in revealing the height differences in low and high pressures.

When short and tall persons are compared in specific weight groups, we find a complete reversal of the distribution of high pressures. In all three weight groups, light, middle, and heavy, short men and women have a higher incidence of high systolic and diastolic pressures than tall men and women. In the distribution of low pressures we found no difference between short and tall men and women in any weight group.

DISCUSSION

In our previous studies of 10,883 persons blood pressure was found to be correlated to both body build and obesity. However, since the literature is almost unanimous in stating that height does not affect blood pressure, no study of this

relation was contemplated. Almost accidentally, in a study of body build, it was discovered that the lateral person is most often of short stature. Since hypertension is most common in the lateral build it was reasoned, therefore, that height must show some effect on blood pressure. Accordingly, this study was made of the relation of height to blood pressure, and statistical breakdowns proved our reasoning to be true. The short person had higher mean and modal pressures and a higher incidence of high pressure than the tall person in the total group.

Since we have shown that the lateral build person of any height group is more susceptible to hypertension, it is obvious that the higher mean and modal pressure of the short group is a distortion due to a preponderance of lateral builds among short men. This could be corrected only by holding the build groups constant and then comparing the incidence of high and low pressures between short and tall persons.

When such a study was made of the blood pressure distribution within the various build-height groups, an entirely new and interesting height variation was discovered. The tall lateral person had a higher mean and modal pressure than the short lateral person, and a much greater incidence of hypertension. The tall lateral person had a smaller chance of having a low pressure than did the short lateral person. The actual to expected ratio further confirmed this striking finding. We, therefore, have an apparent contradiction in the influence of height on blood pressure. The true height difference is the greater hazard of hypertension carried by tall broad persons. The reversed finding in the total group showing the short persons to have more hypertension than the tall persons is due to the greater incidence of lateral build in the short group. Thus there is really no contradiction. The influence of build on hypertension is again strongly emphasized.

When the group was broken down into specific weight classifications, the short group was shown to have a higher incidence of hypertension than the tall group. This difference held true in all three weight groups. Height, therefore, influences blood pressure in two opposing ways. When the build is held constant, the tall person is more susceptible to hypertension. When the weight is held constant, the short person has the greater incidence of hypertension. How can these two divergent facts be reconciled?

The difference between tall and short in the various build groups is the true constitutional factor influencing blood pressure. This represents the morphologic and biochemical susceptibility to hypertension of the tall broad type, the exact nature of which requires further study. The pressure difference between tall and short men and women in the specific weight groups is dependent upon the greater incidence of lateral build in the short group. For, as we shall show in a subsequent study, obesity is linked to the lateral build. Therefore, we are really measuring to a great degree the lateral build factor when we study blood pressure in the short overweight person.

This finding, that hypertension is a greater hazard of the tall lateral person, suggests a comparison with Draper's[15] contention that the women with hyperten-

sion ". . . are the tallest of all women. . . ." Draper does not make the same statement about men; nevertheless, our study surely confirms Draper's finding and probably holds for both men and women.

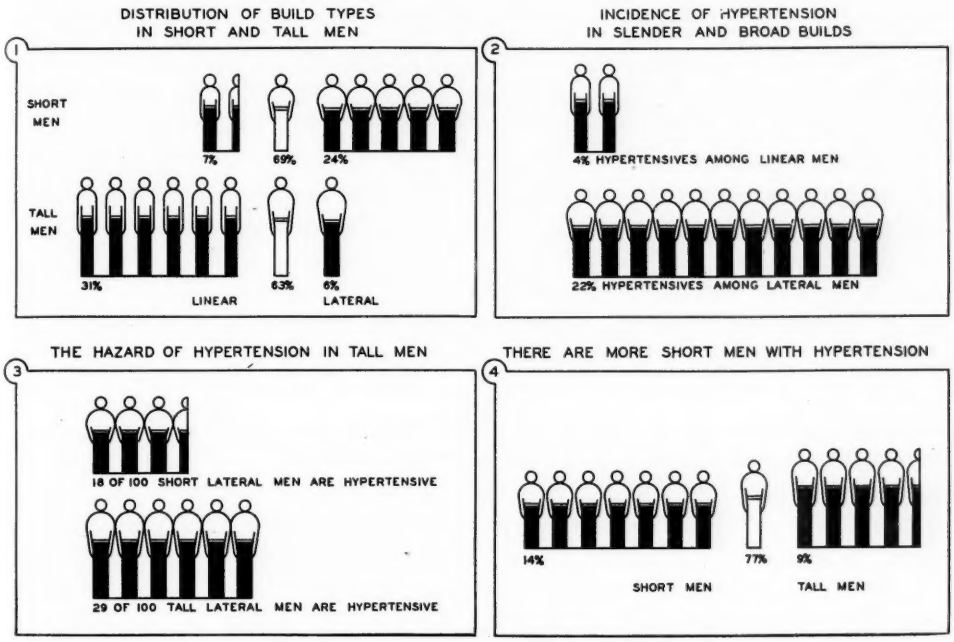

Fig. 5.—This chart summarizes the important relationship of height and build to blood pressure. (1) In a random group there are more lateral or broad builds among short persons than among tall. (2) It is shown that hypertension is more frequently found among the lateral build individuals of any height. (3) There is a basic difference between short and tall men in their susceptibility to hypertension. Tall lateral men have greater hypertension than short lateral men. (4) It is, therefore, clear that although the tall person shows the greater hazard of hypertension, we find more short men with hypertension because of the greater incidence of broad builds among short persons.

The analysis of the distribution of hypertension furnishes a partial explanation of the decrease in incidence of tall men in the older ages as pointed out earlier in this paper. The greater hazard of hypertension among the tall broad men should cause an increase in their mortality in the earlier age groups. Costanzo[16] says that "as the age at death increased, body structure became more and more short shaped." However, the vast number of short hypertensive persons should distort the mortality records to show a much higher death rate among short men. The Medico-Actuarial Mortality Investigation[17] confirms this supposition in the statement that "at the younger ages tall men have proved less desirable risks than short men. At the older ages the short men . . . have been slightly worse risks than tall men." Dublin[14] makes the same statement for women but adds in another article[18]: "Extremes of stature [both short and tall] are decided handicaps."

The predilection of hypertension for lateral build has also made its mark on the mortality, as recorded by chest measurements. As Dublin[19] states, ". . . The lower mortality is shown in the chest-girth groups below the mean." He implies further that in any height group the smallest chest has the lower mortality. Since hypertensive heart disease plays such a large part in crude mortality, it would

seem that much of the differential mortality of height groups can be explained on the basis of the hazard of hypertension among lateral build persons and the greater proportion of lateral build in the persons of short stature.

CONCLUSIONS

1. A gross anthropologic study of 2,552 men and 2,021 women shows for the first time in medical literature the positive correlation of height to blood pressure.

2. Blood pressure is shown in this study to be affected by height. A review of the literature shows that no study, thus far, has found the height difference as reported in this paper. An explanation for this failure is probably due to a lack of correct statistical delineation of build types and the exclusive reliance upon mean and modal pressures, instead of a study of the incidence of low and high pressure distribution and the actual to expected ratio.

3. The incidence of tall men and women decreased steadily with an increase in age. This is an unusual and striking phenomenon, and is probably due chiefly to a high mortality of tall persons in earlier age groups.

4. In a random group of mixed builds and weights short men and women will show higher mean and modal systolic and diastolic blood pressures than tall men and women. Short men and women showed a higher incidence of high pressures than tall men and women.

5. When the build groups are separated and held constant, a marked difference in blood pressure is noted between tall and short persons and is reversed to the height relationship mentioned above.

6. The tall lateral or broad individual is more susceptible to hypertension than the short lateral one and is less likely to have a low pressure.

7. Tall lateral or broad men show an actual to expected ratio of about two and one-half times as many systolic and diastolic hypertensives as short lateral men.

8. Tall lateral women show twice as many systolic and diastolic hypertensives as short lateral women.

9. Tall lateral women show less than one-third the low systolic and diastolic pressures as short lateral women.

10. Tall lateral men have a slightly smaller incidence of low pressure than short lateral men.

11. The actual to expected ratio shows that among linear or thin men the tall men have only one-half as many low systolic and diastolic pressures as the short men.

12. The actual to expected ratio shows no difference between short and tall linear or thin women of low and high pressures.

13. In a previous paper it was shown that the lateral or broad build individual in any height group carried the highest incidence of hypertension. In this paper it is shown that lateral build is most often found among short men and women.

14. This unequal distribution of lateral build in short and tall persons explains the discrepancy mentioned in conclusion number 5. Although the tall person carries a greater hazard of hypertension than any other individual, the

larger number of lateral builds among short persons causes the bulk of the hypertensive population to be found among short persons.

15. The short individual shows a higher incidence of hypertension than the tall one in any weight group.

16. There are, therefore, three influencing height factors in hypertension. First, there is the difference in blood pressure between tall and short persons in specific build groups. This is purely a height difference. Second, there is the difference in pressure between tall and short persons when weight is held constant. Third, there is the difference in pressure between short and tall persons in any total group in which build and weight are naturally mixed. The second and third height differences are dependent upon the build factor.

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STUDIES ON EXPERIMENTAL LESIONS IN THE KIDNEYS OF RABBITS*

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THE purpose of this paper is to report the results of investigations on the production of experimental renal lesions in rabbits by homologous nephrotoxins and certain bacterial filtrates. The work of Shwartzman,[1, 2] Gerber,[3] and Apitz[4] indicates that it is possible to damage rabbit kidneys by two or more injections of selected bacterial filtrates into the systemic circulation. Masugi[5] showed that experimental lesions could be produced in the kidneys of rabbits by injection of duck anti-rabbit kidney serum. Smadel[6-9] has reported similar results in rats by the use of rabbit anti-rat kidney serum. These lesions bore a striking similarity to those of human glomerulonephritis. To get a potent anti-kidney serum it was necessary to use two species of animals. Schwentker and Rivers[10] showed that repeated injections of autolyzed rabbit brain into rabbits produced complement-fixing antibodies. This finding suggested that by autolyzing homologous protein, one could render it antigenic to the same species of animal. An attempt was made, therefore, to autolyze renal protein by tying off the renal artery of a rabbit's kidney and allowing it to degenerate in vivo. In the experiments to be reported rabbits were injected with emulsions of such degenerated kidney and their serum was used in an attempt to produce renal changes in other rabbits.

Other experiments were conducted in an attempt to damage renal proteins by a Shwartzman reaction.

EXPERIMENTS ON DEGENERATED RABBIT KIDNEY

Under ether anesthesia the left renal artery of a rabbit was ligated. Eighteen hours after ligation the rabbit was killed and both kidneys were removed aseptically. The left kidney was definitely swollen and discolored. The normal right kidney was perfused through the renal artery with sterile normal saline in order to remove the blood remaining in it at the time of death. No attempt was made to perfuse the degenerated kidney. Each kidney was then cut up aseptically with scissors and macerated in a mortar. Sterile emulsions of each were made by shaking up the macerated kidneys with 50 c.c. of sterile saline. Two rabbits then received seven intraperitoneal injections each of the two emulsions at daily intervals and were killed by bleeding to death. The blood from each rabbit was preserved carefully. On autopsy neither of these injected animals showed significant pathologic changes in the kidneys or other organs.

The precipitin titer of the blood of these two injected rabbits was examined by means of Wright's capillary tube technique. It was found that precipitating

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antibodies to the dilutions of the ischemic kidney emulsion were present in significant amounts in the blood of the rabbit that had received that emulsion. The blood of the control rabbit that had received injections of normal kidney emulsion contained no precipitating antibodies.

Two other rabbits were subjected to intravenous injections of the precipitating rabbit serum. One received a single intravenous dose of 3 c.c. of serum and was killed nineteen hours later. Autopsy revealed no pathologic change in the kidneys or other organs. A second rabbit received six intravenous injections of the precipitating serum daily and was killed thirty-two hours after the last injection. On autopsy the kidneys showed no significant lesions. It was concluded from these experiments that, although the serum injected contained precipitating antibodies, there was no evidence of its being nephrotoxic in the sense of producing nephritis.

EXPERIMENTS WITH BACTERIAL FILTRATES

The bacterial filtrates used in these experiments were prepared from cultures of typhoid bacilli and hemolytic streptococci. Typhoid organisms were from a stock culture and the filtrates were prepared by the method of Shwartzman. These bacteria were grown on large surfaces of agar and washed off with normal salt solution. The washings were centrifuged and the supernatant fluid was passed through a Berkefeld filter. The resulting filtrate was used for the animal injections. In earlier experiments the streptococci used were obtained from an infected human knee joint. Those used in the last group of experiments were a group A hemolytic strain obtained through the kindness of Dr. Ronald Hare, of the Connaught Laboratories, University of Toronto. In both cases they were grown in broth and filtered through Berkefeld candles.

The technique of injection of the left renal artery was as follows: Under ether anesthesia a longitudinal lumbar incision was made. The lumbar abdominal muscles were split in gridiron fashion, and the artery and vein were exposed without perforating the peritoneum. Both vein and artery were freed of connective tissue, and a ligature was passed around the vein but not tied. The artery was elevated on a hook at the same time the vein was temporarily occluded by traction on the venous ligature. The artery was injected then by using a syringe and a bent needle. The volume of fluid injected was usually 2 c.c. A successful injection of the artery was evidenced by a distinct pallor of the kidney which could be seen through the peritoneum. The needle then was withdrawn and the hook occluding the artery was removed. Hemorrhage from the puncture in the arterial wall was controlled by a sponge soaked in saline at a temperature of about 44° C. The vein was kept occluded for five minutes in order to prevent the injected fluid from being washed out of the kidney immediately. The venous ligature was removed then and the circulation within the kidney allowed to reestablish itself. The kidney, which was blue and tense at the moment of release, would resume quickly its normal chocolate brown color and become much softer to the touch. The muscles of the loin were repaired and the wound was closed. Rabbits so treated showed no systemic ill effects.

EXPERIMENTAL RESULTS

(1) *Intra-Arterial Streptococcal Filtrate Alone.*—One rabbit received an injection of 1.5 c.c. of the first streptococcal filtrate in its left renal artery. No further injections were made, and the rabbit was killed twenty-nine hours later. Macroscopically the left kidney was slightly paler than the right, but microscopically no significant changes could be found.

(2) *Intra-Arterial Streptococcal Filtrate Followed by Single Intravenous Streptococcal Filtrate.*—The left renal artery of a rabbit was injected with 1.5 c.c. of the same streptococcal filtrate. In twenty-four hours it was given an intravenous injection of 1.5 c.c. of this streptococcal filtrate in the ear vein; the rabbit was killed four hours later. On autopsy the left kidney was paler than the right. Microscopically neither kidney showed any definite abnormality.

(3) *Intra-Arterial Typhoid Filtrate Followed by Single Intravenous Streptococcal Filtrate.*—The left renal artery of a rabbit was injected with 1.5 c.c. of the first typhoid filtrate. Twenty-four hours later an injection of 3 c.c. of the first streptococcal filtrate was made in the ear vein. The animal was killed five hours later. On autopsy the left kidney was pale and swollen while the right kidney was normal. The left kidney weighed 15.5 Gm., and the right kidney 11.5 Gm. Microscopically the left kidney showed little pronounced change in the glomeruli. Surrounding the capsule of a number of glomeruli was a zone of hyaline material which contained no nuclei; it stained pink with hematoxylin and eosin and red with Heidenhain's azocarmine aniline blue. There was disintegration of many proximal tubules with disappearance of nuclei, and similar damage was observed in some of the distal convoluted tubules. Many tubules down to the pelvis were seen to contain hyaline material similar to that described as surrounding some of the glomerular capsules. In this kidney there was damage to the tubules with exudation within as well as outside the nephron. No significant microscopic changes were observed in the right kidney. The liver showed fatty degeneration.

A second rabbit of this group received an injection of 2 c.c. of the second typhoid filtrate in its left renal artery. Twenty-four hours later it was given an intravenous injection of 2 c.c. of the first streptococcal filtrate in the ear vein. It died in about twenty-six hours. In the gross and microscopically both kidneys were normal.

(4) *Intra-Arterial Typhoid Filtrate Followed by Single Intravenous Typhoid Filtrate.*—A rabbit received 2 c.c. of the first typhoid filtrate in its left renal artery and 0.25 c.c. in the skin of the abdomen. At the end of twenty-four hours 3 c.c. of this typhoid filtrate were injected into the ear vein. The animal was killed five hours later. On autopsy the left kidney was slightly pale, and the skin showed no significant erythema. Microscopically some patchy areas of ischemic atrophy were seen in the left kidney. The right kidney was normal. No change was observed in either kidney that might not have been quite spontaneous in the rabbit.

A second rabbit of this group likewise was injected in its left renal artery with 2.5 c.c. of the second typhoid filtrate. At the same time an injection of 0.5 c.c. was made in the skin. Twenty-four hours later, at the time when the skin

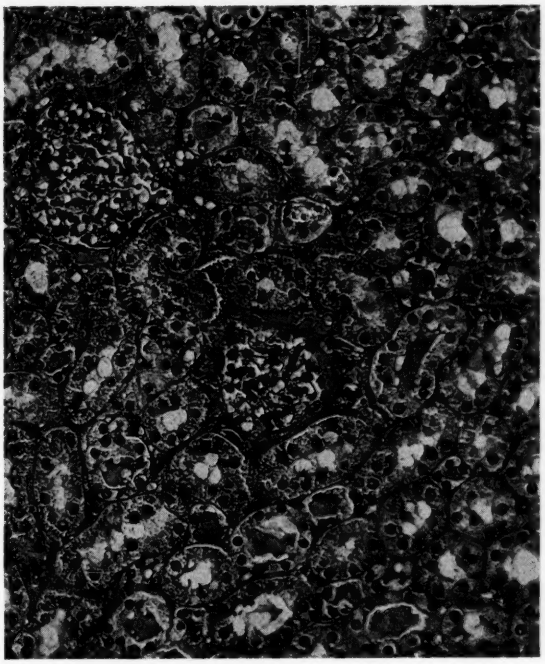

Fig. 1.—First rabbit of Experiment 3. Left kidney showing exudate about glomeruli and in some tubules.

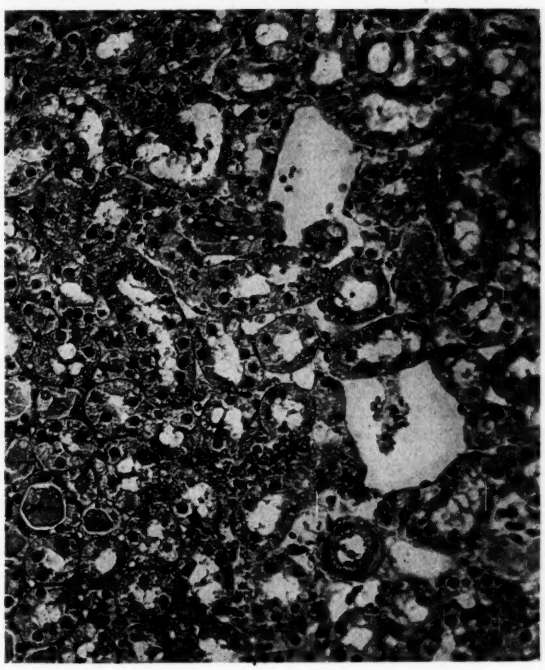

Fig. 2.—First rabbit of Experiment 3. Left kidney showing slightly damaged proximal tubules.

showed a slight erythema, an injection of 2 c.c. of the same second typhoid filtrate was made in the ear vein. The rabbit died three hours later. On autopsy the kidneys showed nothing of note.

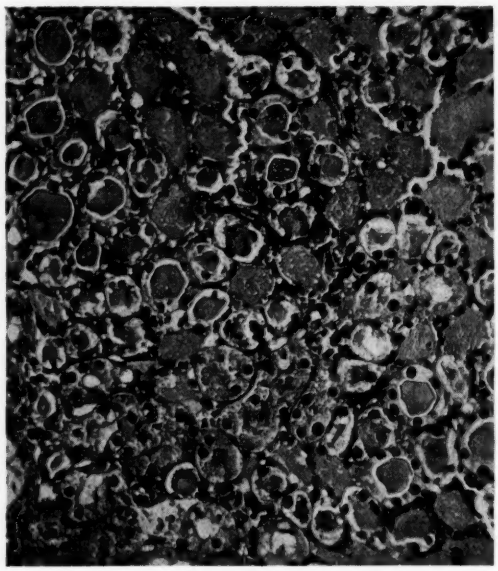

Fig. 3.—First rabbit of Experiment 3. Left kidney showing loop tubules containing exudate.

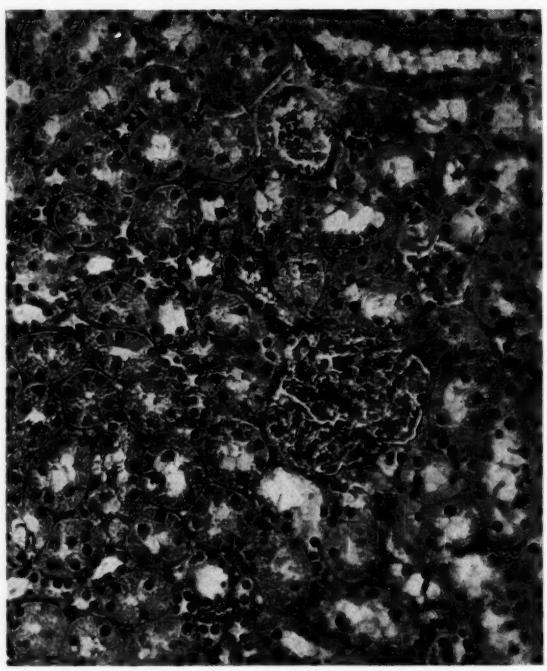

Fig. 4.—First rabbit of Experiment 3. Untouched right kidney—undamaged.

(5) *Intra-Arterial Typhoid Filtrate Followed by Multiple Intravenous Streptococcal Filtrate Injections.*—A single rabbit was given 2 c.c. of a third typhoid filtrate in the left renal artery. Twenty-four hours later it received

an intravenous injection of 2 c.c. of the second streptococcal filtrate. These intravenous streptococcal filtrate injections were repeated once daily until 27 in all had been given. The rabbit was then killed. On autopsy the left kidney appeared pale and granular, and on section had a rather narrow cortex. The right kidney was normal. On microscopic section the left kidney showed marked focal fibrous atrophy. There were no glomerular lesions of note. The right kidney was normal microscopically. The lesions in the left kidney may have been due to the production of thromboses at the time of the arterial injection.

(6) *Intra-Arterial Typhoid Filtrate Followed by Multiple Intravenous Typhoid Filtrate Injections.*—A rabbit was given an injection of 2 c.c. of the third typhoid filtrate in its left renal artery. Twenty-four hours later it received 2 c.c. of the same filtrate in the ear vein, and thereafter daily intravenous injections of 2 c.c. of the same filtrate until 16 in all had been given. The rabbit was killed. On autopsy the left kidney was pale and had a large infarct. The right kidney was normal. Microscopically, apart from the infarct, there was much focal fibrous atrophy of the left kidney. The right kidney was normal.

(7) *Intra-Arterial Typhoid Followed by Multiple Intravenous Streptococcal Injections, Using Controls and Varying Time Intervals.*—Nine rabbits were used in the experiment subdivided into three groups of three. The first two groups were controls and the third the experimental group. The rabbits of the first group each received a single injection of 2 c.c. of the fourth typhoid filtrate in their left renal arteries. The rabbits of the second group each received daily intravenous injections of 2 c.c. of streptococcal filtrate but no antecedent arterial injections. The rabbits of the third, or experimental, group each received an injection of 2 c.c. of the typhoid filtrate in the left renal artery followed by daily intravenous injections of 2 c.c. of streptococcal filtrate. The first rabbit of each group was killed ten days after the initial injection, the second twenty days, and the third thirty days after that injection. The results were disappointing. No significant changes, gross or microscopic, were produced in the kidneys of any of the animals. Minor changes, such as focal fibrous atrophy, edema, and interstitial eosinophilic infiltration, were observed, but nothing that does not occur spontaneously in rabbits. It would appear that the treatment accorded these rabbits, control or experimental, was without effect in producing glomerular changes. Certainly nothing of the nature of glomerulonephritis was observed.

SUMMARY AND CONCLUSIONS

An attempt was made to produce pathologic changes in the kidneys of rabbits by intravenous injections of serum from rabbits that had been treated with intraperitoneal injections of degenerated rabbit kidney emulsion. An attempt was also made to damage rabbit kidney by bacterial filtrates. The results of these investigations were essentially negative. One interesting observation, from a serologic point of view, was the finding of precipitating antibodies in the serum of a rabbit that had been injected intraperitoneally with emulsions of degenerated rabbit kidney. Such precipitating antibodies could not be demonstrated in the blood of a rabbit that had received injections of a normal kidney emulsion. The renal lesions encountered in one rabbit described, while interest-

ing, could not be reproduced. The interpretation of the changes in this single rabbit must remain an open question. The possibility of an allergic reaction may be considered, but it could not be denied that a circulatory disturbance in handling the kidney might have been responsible.

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DISTURBANCES IN THE BLOOD FOLLOWING EXPOSURE TO BENZOL*

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INTRODUCTION

THE fact that benzol when taken into the human body may have deleterious effects upon the hematopoietic system has been known for about forty years.[1] With the exception of lead, there is probably no industrial poison better known to physicians than benzol. A rather extensive literature on the subject of benzol poisoning has accumulated, and most of the reports, especially the more recent ones, contain some mention of the blood picture. The most extensive review of the subject yet to appear is that of the British Industrial Health Research Board.[2] Another comprehensive review is that of Hamilton,[3] published in 1931. The present status of knowledge concerning the blood picture in chronic benzol poisoning is well summed up in the following lines taken from the British report just mentioned: "While the most characteristic blood picture in benzol poisoning remains that described by the earlier authors, a leucopenia with neutropenia, thrombopenia and some anemia, it becomes increasingly evident that many cases present wide variations on this picture."

Most cases of benzol poisoning described in the literature have been fatal or at least of such severe degree that hospitalization has been necessary. Cases of this type are frequently complicated by hemorrhage, infection, or some other secondary factor. Whereas acute poisoning has been well differentiated from the chronic form, little distinction has been made between mild and severe forms of chronic poisoning. Relatively few observers have had an opportunity to study more than two or three isolated cases of chronic benzol poisoning, and not many of these have included a complete set of hematologic observations in their studies. Incomplete observations by a large number of physicians using different methods and terminologies and describing small numbers of cases must result in some confusion.

In the literature dealing with industrial poisoning, control studies are practically never mentioned. Benzol poisoning is no exception to this general rule. A careful observer would never consider publishing results of experimental poisoning in laboratory animals without having performed control experiments, even though "normal" values for the species of animal were supposed to be well known. In industrial poisoning the experimental animal is a human being. It seems entirely illogical to neglect establishing normals for the apparatus and methods in the hands of the particular individual who is making the study.

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This paper presents the results of comprehensive hematologic studies on a large group of individuals who had been exposed to benzol. The significance of the results is enhanced by the inclusion of controls.

CASES STUDIED

The control group used in this investigation consisted of 81 male industrial workers employed in a factory where no occupational hazard could be demonstrated. These workers varied in age between 20 and 65 years. None gave a history of abnormal symptoms and none had any significant abnormal findings on physical examination. For all practical purposes the group could reasonably be considered to be a small but representative sample of the industrial population employed in a nonhazardous industry in New York City. A control series in the industry in which the benzol poisoning occurred was not possible, since no branch of that particular work is free of toxic materials.

TABLE I

TESTS PERFORMED AND NUMBER OF INDIVIDUALS SUBJECTED TO EACH TEST

BLOOD DETERMINATION	CONTROL	BENZOL	TOTAL
Hemoglobin	81	235	316
Erythrocytes	81	159*	240
Leucocytes	81	332*	413
Differential	81	112	193
Platelets	50	107	157
Reticulocytes	47	103	150
Bleeding time	52	105	157
Coagulation time	52	99	151
Sedimentation rate	59	104	163
Hematocrit	81	104	185
Mean corpuscle volume	75	103	178
Mean corpuscle hemoglobin	73	120	193
Mean corpuscle hemoglobin concentration	81	105	186
Fragility of red blood cells	68	92	160
Serum bilirubin	0	102	102

*Thirty-seven erythrocyte counts and 98 leucocyte counts were made by Dr. B. Mintz, of the Division of Women in Industry, of the New York State Department of Labor. I made all other determinations.

The workers exposed to benzol (henceforth referred to as the benzol group) were employed in the rotogravure printing industry in New York City. A full description of this industry and its hazards has been given elsewhere.[4] Suffice it to say here that every individual in the group had been exposed to concentrations of benzol fumes ranging from 11 to 1,060 p.p.m. for a period of at least six months, and in most instances for about three years.* In all, 332 rotogravure workers were examined. A complete set of hematologic observations was made in about 100 cases. The tests performed and the number of individuals subjected to each test are given in Table I. The age distribution of the control and benzol groups is shown in Table II. The differences between the two groups are not so great that they render comparison fallacious.

*The inks used in the particular type of rotogravure printing with which we are concerned contained chemicals other than benzol which might conceivably have produced toxic effects. Since benzol was the predominant solvent and since the effects were compatible with benzol intoxication, the other ingredients are not considered to be of great etiologic significance. The other solvents, all of which were used in relatively small amounts, were toluene, xylene, methyl ethyl ketone, petroleum naphtha, ethyl, butyl, and amyl acetate, and butyl and amyl alcohol.

Since there was no direct relationship between the duration of employment and the nature of the blood findings (Table III), this feature will not be discussed. All the individuals in the benzol group were actually working at the time they were examined. As a result of the blood studies, six were considered poisoned seriously enough to warrant hospitalization. Further studies on these patients have recently been reported.[5] About 100 were referred to various physicians for observation and treatment. All but the six hospitalized persons were allowed to continue to work inasmuch as the use of benzol was discontinued shortly after this study was begun.

TABLE II

AGE DISTRIBUTION*

AGE GROUP	CONTROL		BENZOL	
	NUMBER	PER CENT	NUMBER	PER CENT
20-29 years	33	41.2	154	49.4
30-39 years	12	15.0	86	27.6
40-49 years	21	26.3	52	16.6
50-59 years	8	10.0	15	4.8
60-69 years	6	7.5	5	1.6
Total	80	100.0	312	100.0

*In a few cases the age was not ascertained.

TABLE III

CORRELATION OF LENGTH OF EXPOSURE AND BENZOL EFFECT

The differences in the various exposure groups are not statistically significant (chi square test)

BLOOD PICTURE	TIME OF EXPOSURE IN MONTHS							
	6-12 MO.		13-24 MO.		25-36 MO.		37-60 MO.	
	NUMBER	PER CENT	NUMBER	PER CENT	NUMBER	PER CENT	NUMBER	PER CENT
Normal	24	63.2	22	46.8	97	55.8	34	60.7
Abnormal	14	36.8	25	53.2	77	44.2	22	39.3
Total	38	100.0	47	100.0	174	100.0	56	100.0

METHODS

Erythrocyte and leucocyte counts were performed on capillary blood drawn from the finger tip. Bureau of Standards certified pipettes were used. For hemoglobin determinations the Newcomer method was used and readings were made on a photoelectric colorimeter.* This instrument was considered to offer many advantages, since on certain days as many as 30 or 40 hemoglobin determinations had to be made. Blood smears stained with Wright's stain were used for the differential counts, and a slight modification of Schilling's[6] classification followed. In previous studies on smaller groups of subjects 200 cells were counted. Close agreement was found between the distribution of cells in the first and second hundreds so that in the present study, involving so many subjects, this refinement of technique was not observed.

Reticulocytes were vitally stained on slides previously prepared with a saturated alcoholic solution of brilliant cresyl blue. The number of reticulated cells observed while counting 1,000 erythrocytes was used as the basis for calculating the percentage of reticulocytes. Blood platelets were estimated from stained

*Kindly loaned by the Klett Colorimeter Company, New York.

smears of capillary blood drawn through a drop of 14 per cent magnesium sulfate solution, the smears being stained with Wright's stain. The number of thrombocytes observed while counting 1,000 erythrocytes was used as the basis for calculation.

The bleeding time was determined by Duke's method.[7] Blood was drawn from the lobe of the ear by means of an automatic lancet adjusted to produce a uniform depth of incision. Coagulation time was measured on venous blood by the method of Lee and White.[7] For calculating the sedimentation rate of the erythrocytes the method of Wintrobe and Landsberg[8] was followed and the results were corrected for anemia. A mixture of ammonium and potassium oxalate was used as the anticoagulant. The hematocrit value was obtained in the same tube that was used for the sedimentation rate, and this value was used in calculating the mean corpuscular volume and the mean corpuscular hemoglobin concentration. These indices, as well as the mean corpuscular hemoglobin content, were obtained according to the methods of Wintrobe.[9] To produce maximum packing of the cells, the hematocrit tubes were centrifuged at 3,600 r.p.m. for one hour.* Fragility of the erythrocytes (resistance to hypotonic saline) was tested by the method of Sanford[7] and serum bilirubin was determined as described by Ernst and Förster.[10]

Checking of Results.—In the controls as well as in the benzol group, when results of any test showed decided deviation from the normal, the test was repeated in many instances on a fresh specimen of blood. In a few cases repetition was not possible. Almost invariably the second test agreed closely with the first. Where there was disagreement, the figure nearer to normal was accepted.

NORMAL STANDARDS

In the present study it was not necessary to rely on the figures given in the literature for normal blood values, since a reasonably large control group comprised a part of this investigation. Most of the "normal" groups that have been reported have been made up of students, nurses, and similar types of individuals. Little has been written concerning the "normal" blood picture of industrial workers. There is no reason to believe that standards for workers in nonhazardous industries differ greatly from those of student groups. To support this view, Table IV has been prepared using Osgood's[11] figures for the purpose of comparison. The only significant difference seems to be in the hemoglobin values, there being no obvious reason for the discrepancy other than difference in method. Particular attention is called to the values for lymphocyte percentages. Most writers who have described blood findings in connection with industrial poisoning seem to have accepted the old idea that lymphocytes normally comprise 20 to 30 per cent of the leucocytes. As a result, practically every industrial poison is said to be associated with a relative lymphocytosis. If it is held that any value above 30 per cent of lymphocytes constitutes a lymphocytosis, one is quite certain to find a "lymphocytosis" in a large proportion of

*In previous studies it had been observed that centrifugation at 3,000 r.p.m. for thirty minutes, as recommended by Wintrobe[9], did not always produce constant volume. It is possible that the modification used in the present study was responsible for the fact that the mean corpuscular volume range in the control series was lower than that reported by Wintrobe as normal.

individuals who have been exposed to any industrial poison. Applying the same criterion, about 85 per cent of the cases comprising the normal controls in the present study had a "lymphocytosis." Such a finding suggests the necessity for revising the commonly accepted normal values for lymphocytes to an appreciably higher level. This rather detailed discussion has been included because of the prominent part "relative lymphocytosis" has always played in descriptions of the blood picture of benzol poisoning. The neglect of control blood studies in connection with investigations of industrial poisoning has apparently been responsible for the propagation of an erroneous idea. A recent report by Osgood[12] on the normal differential picture supports the view that the commonly accepted normal range for lymphocytes is far too low.

TABLE IV

COMPARISON OF NORMAL VALUES AS GIVEN BY OSGOOD[11] AND THOSE OF THE CONTROL GROUP IN THE PRESENT INVESTIGATION

	OSGOOD		CONTROL GROUP	
	AVERAGE	RANGE	AVERAGE	RANGE
Red blood cells	5.42 million	4.6-6.2	5.36	4.47-6.3
Hemoglobin	15.8 Gm.	14.0-18.0	14.2	12.2-16.4
Cell volume	44.7 per cent	40-50	45	39-53
Reticulocytes	1.46 per cent	0.5-3.0	0.6	0.2-1.6
White blood cells	7,400	4,500-11,500	8,400	5,500-14,500
Polymorphonuclear cells—young	0.8 per cent	0-5	3.9	0-10
adult	54	33-78	51	27-69
Lymphocytes	38	18-65	37	18-61
Monocytes	4	0-9	5.6	0-16
Eosinophiles	1.9	0-6	2.1	0-9
Basophiles	0.5	0-2	0.4	0-3

TABLE V

DISTRIBUTION OF HEMOGLOBIN VALUES IN CONTROL AND BENZOL GROUPS

HEMOGLOBIN (GM. PER 100 C.C.)	CONTROL		BENZOL	
	NUMBER	PER CENT	NUMBER	PER CENT
Less than 11	0	0	1*	0.4
11.0-11.9	0	0	10	4.3
12.0-12.9	2	2.4	25	10.5
13.0-13.9	25	30.9	95	40.5
14.0-14.9	40	49.5	78	33.2
15.0-15.9	12	14.8	20	8.6
16.0-16.9	2	2.4	6	2.5
Total	81	100.0	235	100.0

*This case had a hemoglobin value of 8.0 Gm.

HEMATOLOGIC OBSERVATIONS ON 332 WORKERS EXPOSED TO BENZOL AND 81 CONTROL SUBJECTS

The results of the blood studies are presented in Tables V to XXI. Comment on the various findings will concern itself primarily with a consideration of the entire benzol group as compared with the control group. A number of cases showing severe damage to the blood-forming organs have been selected for special consideration.

Hemoglobin.—The details of the hemoglobin determinations are set forth in Table V. The most significant point is that 84.8 per cent of the benzol group had hemoglobin values within normal limits. Subnormal amounts of hemo-

globin were found only in those cases which had evidence of severe injury to the hematopoietic system. The results indicate that a low hemoglobin value is not a prominent part of the picture of chronic benzol exposure and in general is present only when the erythrocytes have fallen to a very low level. As will be pointed out later the hemoglobin values remained relatively high in relation to the erythrocytes. In other words, hyperchromia of the red corpuscles was the rule.

TABLE VI

DISTRIBUTION OF ERYTHROCYTE COUNTS IN CONTROL AND BENZOL GROUPS

ERYTHROCYTES (MILLIONS PER C.MM.)	CONTROL		BENZOL	
	NUMBER	PER CENT	NUMBER	PER CENT
1.5-1.99	0	0	1	0.6
2.0-2.49	0	0	1	0.6
2.5-2.99	0	0	3	1.9
3.0-3.49	0	0	3	1.9
3.5-3.99	0	0	22	13.9
4.0-4.49	2*	2.4	46	29.0
4.5-4.99	16	19.8	50	31.4
5.0-5.49	33	40.8	29	18.2
5.5-5.99	28	34.6	4	2.5
6.0-6.49	2	2.4	0	0
Total	81	100.0	159	100.0

*These two counts were 4.47 and 4.48 millions, respectively.

Erythrocytes.—Details of the erythrocyte counts are given in Table VI. Whereas but two individuals in the control group fell below 4.5 million (and these two but very slightly below), 76 workers, or 47.9 per cent, of those exposed to benzol whose red blood cells were counted had subnormal erythrocyte values. It is obvious, therefore, that a diminution in red corpuscles is a relatively frequent finding in chronic benzol exposure. The polycythemia described by certain observers,[13, 14] in early benzol poisoning was not found in the present series. It is possible that if erythrocyte counts had been done on the entire group, some higher counts might have been encountered. On the other hand, since the individuals studied had been exposed from six to sixty months to concentrations of 11 to 1,060 p.p.m., they may reasonably be said to represent all degrees of benzol effect, ranging from none at all to severe damage. It would seem that polycythemia, if it occurs in benzol exposure, should have appeared in at least a few members of the benzol group.

Qualitative changes in the erythrocytes, such as poikilocytosis, basophilic stippling and nucleated forms, were seen only in cases in which the degree of anemia was severe. All the severely anemic cases, however, did not exhibit these qualitative abnormalities. No effort was made to estimate the size of the red corpuscles from stained blood films, since it was felt that simple inspection is not entirely reliable and use of the Price-Jones technique was too time-consuming for a series as large as the present one. The average size of the erythrocytes (mean corpuscular volume) was calculated according to the method of Wintrobe.[9] As will be shown later there was a definite tendency to macrocytosis in the benzol group.

Leucocytes.—As was stated in the introductory remarks, a leucopenia has always been considered one of the most characteristic features of benzol poison-

ing. If one considers only cases of an extreme degree of poisoning, this view is undoubtedly correct. If, however, reliance is placed on the finding of leucopenia to diagnose mild or early cases, serious error might result. In this connection Hamilton[15] has stated that "if the physician depends on a leucopenia for the diagnosis and fails to make a count of the red cells, the patient may be fatally poisoned before he discovers that benzol poisoning has caused marrow aplasia." It can be seen in Table VII that only 14.5 per cent of the benzol group had less than 5,000 white blood cells and 69 per cent had more than 6,000 leucocytes. It might be argued that those patients having normal white cell counts were not really poisoned, but to sustain such a point of view one would have to contend that anemia, macrocytosis, and thrombocytopenia were no evidence of benzol poisoning.

TABLE VII

DISTRIBUTION OF LEUCOCYTE COUNTS IN CONTROL AND BENZOL GROUPS

LEUCOCYTES (PER C.MM.)	CONTROL		BENZOL	
	NUMBER	PER CENT	NUMBER	PER CENT
2,000- 2,999	0	0	6	1.8
3,000- 3,999	0	0	16	4.8
4,000- 4,999	0	0	26	7.9
5,000- 5,999	7	8.6	55	16.5
6,000- 6,999	19	23.4	58	17.5
7,000- 7,999	16	19.8	55	16.5
8,000- 8,999	11	13.6	48	14.6
9,000- 9,999	10	12.4	24	7.2
10,000-10,999	13	16.1	26	7.9
11,000-11,999	3	3.7	7	2.1
12,000-12,999	1	1.2	4	1.2
13,000-13,999	0	0	3	0.9
14,000-14,999	1	1.2	2	0.6
15,000-15,999	0	0	0	0
16,000-16,999	0	0	2	0.6
Total	81	100.0	332	100.0

It has occasionally been stated that leucocytosis is found in early benzol poisoning. The incidence of high white blood cell counts in the present series was not sufficiently frequent to support the view that leucocytosis is a characteristic feature in benzol exposure.

Lymphocytes.—Mention has already been made of the frequency with which relative lymphocytosis has been described in connection with industrial poisoning in general and benzol poisoning in particular. It is felt that the commonly accepted normal range of lymphocytes is far too low and that this misconception of what is normal has resulted in a certain amount of confusion. Inspection of Table VIII shows that lymphocyte percentages above 40 occurred in more than one-third of the control group, and that values above 50 per cent of lymphocytes were by no means rare. Further, only 12.4 per cent of the control group had between 20 and 30 per cent of lymphocytes. It has been pointed out in Table IV that the figures for range and average of this control group were very close to those given by Osgood[11, 12] as his normals.

On comparing the benzol group with the control group (Table VIII) perhaps the most striking feature is that the incidence of lymphocyte values between 20 and 30 per cent, the traditional normal, was nearly three times as great in

TABLE VIII

DISTRIBUTION OF LYMPHOCYTE PERCENTAGES IN CONTROL AND BENZOL GROUPS

LYMPHOCYTES (PER CENT)	CONTROL		BENZOL	
	NUMBER	PER CENT	NUMBER	PER CENT
10-19	2	2.4	10	8.9
20-29	10	12.4	41	36.6
30-39	40	49.5	41	36.6
40-49	23	28.3	15	13.4
50-59	5	6.2	5	4.5
60-69	1	1.2	0	0
Total	81	100.0	112	100.0

TABLE IX

DISTRIBUTION OF ABSOLUTE LYMPHOCYTE VALUES IN CONTROL AND BENZOL GROUPS

LYMPHOCYTES (ABSOLUTE NUMBERS)	CONTROL		BENZOL	
	NUMBER	PER CENT	NUMBER	PER CENT
750-1,000	0	0	7	6.2
1,000-1,999	9	11.1	54	48.3
2,000-2,999	34	42.0	39	34.8
3,000-3,999	20	24.8	8	7.1
4,000-4,999	14	17.2	4	3.6
5,000-5,999	3	3.7	0	0
6,000-6,100	1	1.2	0	0
Total	81	100.0	112	100.0

TABLE X

DISTRIBUTION OF MONOCYTE AND EOSINOPHILE PERCENTAGES IN CONTROL AND BENZOL GROUPS

A

MONOCYTES (PER CENT)	CONTROL		BENZOL	
	NUMBER	PER CENT	NUMBER	PER CENT
0-5	39	48.2	55	49.1
6-10	39	48.2	49	43.7
More than 10	3	3.6	8	7.2
Total	81	100.0	112	100.0

B

EOSINOPHILES (PER CENT)	CONTROL		BENZOL	
	NUMBER	PER CENT	NUMBER	PER CENT
0-3	64	79.0	82	73.2
4-7	15	18.5	30	26.8
More than 7	2	2.5	0	0
Total	81	100.0	112	100.0

TABLE XI

DISTRIBUTION OF PLATELET COUNTS IN CONTROL AND BENZOL GROUPS

PLATELETS (PER C.MM.)	CONTROL		BENZOL	
	NUMBER	PER CENT	NUMBER	PER CENT
14,000- 50,000	0	0	12	11.2
50,000- 99,000	0	0	23	21.5
100,000-149,000	9	18.0	31	28.9
150,000-199,000	11	22.0	20	18.7
200,000-249,000	20	40.0	12	11.2
250,000-299,000	8	16.0	6	5.6
300,000-380,000	2	4.0	3	2.8
Total	50	100.0	107	100.0

the benzol group as in the control group, whereas values above 40 per cent were twice as frequent in the controls. These findings can lead to but one conclusion, namely, that exposure to benzol produces, if anything, a relative *lymphopenia*. That this lymphopenia is absolute as well as relative is shown in Table IX. It will be pointed out later that in the present study relative lymphocytosis was not found even in those cases which showed evidence of severe damage to the hematopoietic system. It would seem that a frank relative lymphocytosis occurs with some frequency only in those cases of benzol poisoning which terminate fatally with a fully developed picture of aplastic anemia.

Differential Formula.—Except for the lymphocytes, no features in the differential formula require any extended comment. The neutropenia, so prominently featured in the literature, is based on the same questionable standards as the alleged lymphocytosis and so requires no further discussion. In performing the differential counts by Schilling's[6] method, it was apparent that there was no significant shift of the polymorphonuclear cells either to the left or to the right. For this reason, there seemed to be no indication for doing Cooke or Arneth counts.

As can be seen in Table X monocytosis and eosinophilia did not occur in the benzol group nor were unusual numbers of basophiles found. Myeloblasts or myelocytes were not seen in any of the blood smears that were examined.

Blood Platelets.—A reduction in the number of blood platelets was a frequent and significant finding in the group exposed to benzol. Table XI shows that 32.7 per cent of the benzol group had less than 100,000 platelets, while only 19.6 per cent had counts above 200,000. In the control group there were no counts under 100,000, and 60 per cent were above 200,000. The difference between the two groups was marked enough to indicate that a diminution in thrombocytes is an important part of the blood picture resulting from benzol exposure.

TABLE XII

DISTRIBUTION OF RETICULOCYTE PERCENTAGES IN CONTROL AND BENZOL GROUPS

RETICULOCYTES (PER CENT)	CONTROL		BENZOL	
	NUMBER	PER CENT	NUMBER	PER CENT
0-0.5	16	34.1	40	38.8
0.6-1.0	28	59.5	38	36.9
1.1-2.0	3	6.4	22	21.4
More than 2.0	0	0	3*	2.9
Total	47	100.0	103	100.0

*The highest value was 2.4 per cent.

Reticulocytes.—As can be seen in Table XII the reticulocyte counts tended to be slightly higher in the benzol group than in the control group. In general, the higher values were found in those individuals who showed evidence of the most severe degree of damage to the hematopoietic system. No really significant increase in reticulocytes was encountered in the entire series.

Bleeding Time.—Table XIII shows some tendency toward prolongation of the bleeding time in the benzol group, but outspoken abnormality was rare. The two individuals whose bleeding time was longer than fifteen minutes had 46,000

and 30,000 blood platelets, respectively. These were by no means the lowest platelet counts found in the series. Of particular interest is the fact that although in 12 cases thrombocytes numbered less than 50,000, and in 35 cases were below 100,000, only two individuals had excessively long bleeding times, and in only three more was the bleeding time moderately prolonged. It is apparent that following benzol exposure it is possible for the platelets to reach a very low level without any change in the bleeding time.

TABLE XIII

DISTRIBUTION OF BLEEDING TIMES IN CONTROL AND BENZOL GROUPS

BLEEDING TIME (MINUTES)	CONTROL		BENZOL	
	NUMBER	PER CENT	NUMBER	PER CENT
Less than 2	43	82.7	55	52.4
2-4	9	7.3	45	42.8
4.5-6	0	0	3	2.9
More than 6	0	0	2*	1.9
Total	52	100.0	105	100.0

*In these two cases the bleeding was controlled by local pressure after fifteen minutes.

TABLE XIV

DISTRIBUTION OF COAGULATION TIMES IN CONTROL AND BENZOL GROUPS

COAGULATION TIME (MINUTES)	CONTROL		BENZOL	
	NUMBER	PER CENT	NUMBER	PER CENT
Less than 9	10	19.2	29	29.3
9-12	34	65.5	53	53.5
13-16	7	13.4	13	13.1
More than 16	1	1.9	4	4.1
Total	52	100.0	99	100.0

Coagulation Time.—Inspection of Table XIV reveals that no significant difference in coagulation time between the control and the benzol groups was found to exist. In the two cases already mentioned as having prolonged bleeding time, retraction of the clotted blood was delayed and the clot remained friable.

Erythrocyte Sedimentation Rate.—Included in the present studies for the sake of completeness is the erythrocyte sedimentation rate, which has been said to yield abnormal results in a great variety of diseased states. Table XV shows a very close agreement between the results in the control and benzol groups. It may be said that the sedimentation speed of the red corpuscles is probably of no significance in connection with benzol exposure.

TABLE XV

COMPARISON OF SEDIMENTATION RATES OF ERYTHROCYTES IN CONTROL AND BENZOL GROUPS

ERYTHROCYTE SEDIMENTATION RATE (MM. PER HOUR)	CONTROL		BENZOL	
	NUMBER	PER CENT	NUMBER	PER CENT
Less than 9	51	86.5	93	89.4
More than 9	8	13.5	11	10.6
Total	59	100.0	104	100.0

Hematocrit (Cell Volume).—It is not surprising that in the benzol group, where anemia was a frequent finding, the hematocrit values should show a

tendency to be lower than in the control group. Table XVI shows that in the benzol group nearly twice as many individuals were found with cell volumes less than 45 per cent as in the control group, and conversely, twice as many in the control group had values greater than 45 per cent. This difference is probably nothing more than a function of the anemia and has no specificity as far as benzol exposure is concerned.

TABLE XVI

DISTRIBUTION OF HEMATOCRIT VALUES IN CONTROL AND BENZOL GROUPS

HEMATOCRIT (CELL VOLUME %)	CONTROL		BENZOL	
	NUMBER	PER CENT	NUMBER	PER CENT
Less than 40	1	1.2	16	15.4
40-44	31	38.4	59	56.7
45-49	47	58.0	27	26.0
50 or more	2	2.4	2	1.9
Total	81	100.0	104	100.0

Mean Corpuscular Volume (M.C.V.)—As was previously mentioned the mathematical method of measuring the average size of the erythrocytes was considered more suitable for the present study than methods depending on estimates or measurements of cell size in stained blood films. (M.C.V. = hematocrit × 10 ÷ red blood cells in millions.) Apparently this is the first time this method has been applied to a large group of individuals who have been subjected to exposure of the type described in this study. Since the test is simple and well standardized, comparison of subsequent studies with those reported here can reasonably be made.

TABLE XVII

DISTRIBUTION OF MEAN CORPUSCULAR VOLUME VALUES (AVERAGE SIZE OF ERYTHROCYTES) IN CONTROL AND BENZOL GROUPS

MEAN CORPUSCULAR VOLUME (cμ)	CONTROL		BENZOL	
	NUMBER	PER CENT	NUMBER	PER CENT
70- 74	7	9.3	1	0.9
75- 79	9	12.0	2	1.9
80- 84	19	25.4	9	8.8
85- 89	20	26.7	19	18.5
90- 94	13	17.3	24	23.2
95- 99	3	4.0	15	14.6
100-104	4	5.3	16	15.5
105-109	0	0	8	7.8
110-114	0	0	7	6.9
115-119	0	0	2	1.9
Total	81	100.0	103	100.0

The normal range for mean corpuscular volume given by Wintrobe[9] is from 80 to 94 cμ. In the present study 69.4 per cent of the patients in the control group fall within this range, with 21.3 per cent below and 9.3 per cent above. In the benzol group 50.5 per cent fall within Wintrobe's normal range, 2.8 per cent are below and 46.7 per cent are above. The details are given in Table XVII. No matter how the figures are analyzed, no conclusion seems possible except that patients in the benzol group had larger erythrocytes than those in the control group; in other words, the anemia associated with benzol exposure is of the

macrocytic type. The fact that the technique followed for determining mean corpuscular volume was a slight modification of that described by Wintrobe[9] does not invalidate the comparison between the control and benzol groups in this study.

Mean Corpuscular Hemoglobin (M.C.H.).—This method of expressing the average hemoglobin content of the erythrocytes is analogous to color index. It is the preferred method since it is not influenced by varying views as to what constitutes 100 per cent hemoglobin or 100 per cent red corpuscles. (M.C.H. = grams of hemoglobin per 1,000 c.c. of blood ÷ red blood cells in millions.)

According to Wintrobe,[9] the upper limit of normal for M.C.H. is 32 $\mu\mu$g. Table XVIII shows that no case in the control group exceeded this figure, whereas 30.9 per cent of the benzol group had abnormally high M.C.H. values. Further, in the low ranges, 24 to 29 $\mu\mu$g, there were found 87.7 per cent of the controls and only 35.8 per cent of the benzol cases. The findings seem to indicate quite definitely that there is hyperchromia of the erythrocytes of individuals who have been exposed to benzol.

TABLE XVIII

DISTRIBUTION OF MEAN CORPUSCULAR HEMOGLOBIN VALUES (AVERAGE HEMOGLOBIN CONTENT OF ERYTHROCYTES) IN CONTROL AND BENZOL GROUPS

MEAN CORPUSCULAR HEMOGLOBIN ($\mu\mu$G)	CONTROL		BENZOL	
	NUMBER	PER CENT	NUMBER	PER CENT
24-26	30	41.2	12	10.0
27-29	34	46.5	31	25.8
30-32	9	12.3	40	33.3
33-35	0	0	21	17.5
36-38	0	0	11	9.2
39-41	0	0	5	4.2
Total	73	100.0	120	100.0

TABLE XIX

DISTRIBUTION OF MEAN CORPUSCULAR HEMOGLOBIN CONCENTRATION VALUES IN CONTROL AND BENZOL GROUPS

MEAN CORPUSCULAR HEMOGLOBIN (CONC. PER CENT)	CONTROL		BENZOL	
	NUMBER	PER CENT	NUMBER	PER CENT
25-26	1	1.2	0	0
27-29	5	6.1	2	1.9
30-32	55	67.9	40	38.0
33-35	19	23.6	53	50.6
36-38	1	1.2	9	8.6
39	0	0	1	0.9
Total	81	100.0	105	100.0

Mean Corpuscular Hemoglobin Concentration (M.C.C.).—The purpose of performing the mean corpuscular hemoglobin concentration is to ascertain whether hyperchromia is merely a reflection of macrocytosis, or whether it is a result of actual overloading of cells with hemoglobin. (M.C.C. = grams of hemoglobin per 100 c.c. of blood ÷ hematocrit. The result is expressed in per cent.) Wintrobe[9] gives 33 to 38 per cent as the normal range, this being slightly

higher than that of the control group in the present study. If one compares the benzol group with the controls (Table XIX), it is apparent that the great majority in each group fell between 30 and 35 per cent; 91.5 per cent of the controls and 88.6 per cent of the benzol group were in this range. Only one individual in the entire series had an M.C.C. value above Wintrobe's normal limit. Nevertheless, the trend in the benzol group is distinctly higher than that of the controls, and statistical analysis of the two groups (see Table XXII) indicates that there is a significant difference. This would suggest that the hyperchromia associated with benzol exposure is due not only to macrocytosis but also to increased concentration of pigment in the erythrocytes.

Fragility of the Erythrocytes.—The test for fragility of the erythrocytes was performed on 68 controls and 92 members of the benzol group. When the results in the two groups were compared, no significant difference was found. This being the case, the findings are not given in detail.

Serum Bilirubin.—Since the test for serum bilirubin was not performed in the control group, the normal values given by the authors of the method followed[10] will have to serve for comparison. These observers state that a serum bilirubin value greater than 0.75 mg. per 100 c.c. of blood is abnormal. Table XX shows that 32.4 per cent of the workers in the benzol group exceeded this figure, with 10.8 per cent having more than 1.0 mg. per cent.

TABLE XX

DISTRIBUTION OF SERUM BILIRUBIN VALUES IN THE BENZOL GROUPS

SERUM BILIRUBIN (MG. PER CENT)	BENZOL	
	NUMBER	PER CENT
Less than 0.75	69	67.6
0.75-1.0	22	21.6
More than 1.0	11	10.8
Total	102	100.0

Patients Showing Severe Hematologic Abnormality.—In order that some comparison might be made between the present series and those cases which form the basis for most of the reports in the literature, a group of individuals showing severe hematopoietic damage has been selected for special consideration. The group was selected arbitrarily and consists of cases in which any *one* of the following conditions was present: 1. Erythrocytes of 3.0 million or less. 2. Leucocytes of 4,000 or less. 3. Platelets of 50,000 or less.

A composite picture of the more important findings in this group is given in Table XXI. Since these cases have been artificially selected, any extensive analysis of the blood pictures would have little meaning. Among other things, Table XXI reveals the following blood findings:

1. Every case but one showed some degree of leucopenia. In 3 cases (Nos. 9, 12, and 20) the leucopenia was not associated with significant diminution in erythrocytes or thrombocytes.

2. Fifteen of the 23 cases had subnormal red blood cell counts. In all but two of these the white blood cells and platelets were also distinctly subnormal.

TABLE XXI

BLOOD FINDINGS IN THE SEVERELY INVOLVED GROUP

CASE NO.	HB. (GM./100 C.C.)	R.B.C. (MILLIONS)	W.B.C.	LYMPH. (%)	PLATE-LETS	BLEEDING TIME (MIN.)	COAG. TIME (MIN.)	HEMATO-CRIT (C.V. %)	M.C.V.	M.C.H.	M.C.C.	E.S.R.	SERUM BILIRUBIN (MG. %)
1	13.7	3.51	4,650	34	49,000	2	11	40	114	39	34	25	0.84
2	13.2	3.97	3,800	28	159,000	1	8	41	104	33	32	2	1.02
3	15.1	5.19	3,750	28	62,000	1	8	41	79	29	37	4	0.93
4	11.7	2.94	2,650	29	38,000	0.5	9	30	103	40	39	1	1.12
5	8.0	2.49	3,900	34	27,000	1	7	24	97	32	33	4	1.03
6	13.8	4.73	4,550	32	46,000	15	11	42	89	29	33	1	0.93
7	11.7	3.11	3,300	36	28,000	2	7	34	111	38	38	2	---
8	12.7	5.08	4,750	25	50,000	2.5	6	42	83	25	30	1	0.73
9	14.1	4.59	3,750	35	152,000	1.5	5	47	101	31	30	1	0.73
10	13.7	3.69	4,200	24	14,000	6	-	38	104	37	36	2	---
11	11.2	3.20	3,100	36	59,000	2	8.5	31	97	35	36	16	0.87
12	14.1	4.98	3,700	23	149,000	-	-	--	--	29	--	-	---
13	14.5	4.24	3,150	36	34,000	4	12	46	109	34	31	6	0.57
14	12.6	3.90	4,000	53	43,000	3.5	15	36	92	32	35	6	0.63
15	14.8	4.56	5,050	41	50,000	2.5	12	46	101	32	32	1	0.97
16	11.4	2.50	2,750	42	30,000	15	11	30	120	46	38	4	0.90
17	15.2	4.59	5,400	42	14,000	2.5	15	41	89	33	37	6	0.66
18	15.3	4.06	4,150	37	36,000	1.5	9.5	46	113	38	33	10	0.68
19	12.7	3.98	3,700	34	56,000	3	11.5	36	90	32	35	2	0.71
20	14.6	4.63	3,900	29	139,000	3	11	42	91	32	35	1	0.71
21	15.1	4.26	11,000	24	26,000	2	10	45	105	35	33	4	0.63
22	13.0	4.47	3,200	30	121,000	1	10	40	90	29	33	2	0.48
23	13.4	3.74	3,800	34	75,000	1	9	40	107	35	33	2	0.52

TABLE XXII

STATISTICAL COMPARISON OF THE CONTROL AND BENZOL GROUPS

The mean values were calculated from frequency distributions and hence vary slightly from those in Table IV, which were calculated directly. The $\frac{x}{\sigma}$ values may be considered to indicate significant differences in all instances.

	CONTROL				BENZOL				DIFF. IN MEANS	σ OF DIFF. IN MEANS	DIFF. / σ OF DIFF.
	NO. OF CASES	MEAN	σ	σ OF MEAN	NO. OF CASES	MEAN	σ	σ OF MEAN			
Hemoglobin	81	14.34	0.7928	0.0881	235	13.87	1.07	0.0698	0.47	0.1122	4.18
R.B.C.	81	5.32	0.4239	0.0471	159	4.48	0.6681	0.530	0.84	0.0707	11.88
W.B.C.	81	8,253	1,896	211	332	7,295	2,368	130	988	247.8	3.86
Lymph. %	81	35.25	11.65	1.29	112	31.79	9.66	0.9128	3.46	1.5838	2.18
Lymph. no.	81	3,142	1,136	126	112	2,036	846	80.0	1,106	149.3	7.4
Platelets	50	208,000	48,590	6,872	107	137,617	73,971	7,150	70,383	9,918	7.09
M.C.V.	81	85.83	7.24	0.8044	103	95.51	9.40	0.9262	9.68	1.2267	7.89
M.C.H.	73	27.6	2.016	0.236	120	31.6	3.759	0.342	4	0.416	9.62
M.C.C.	81	32.02	1.48	0.164	105	33.63	2.07	0.182	1.61	0.247	6.54

3. Thrombocytopenia was present in 18 of the 23 cases. In two of these (Nos. 15 and 17) the erythrocytes and leucocytes were not significantly reduced.

4. The erythrocytes were abnormally large in 14 of the 22 cases in which size was determined.

5. Only one of the 22 cases with leucopenia had even a slight elevation of the lymphocyte percentage.

Actually the lymphocyte figures were lower in the severely involved group than in the control group. Values above 40 per cent occurred in 35.7 per cent of the latter as compared with 17.4 per cent of the former.

DISCUSSION

It would seem superfluous to indulge in any extended discussion of the foregoing findings. Points of interest to the industrial hygienist have received attention in another publication[4] which deals with benzol poisoning as an industrial hazard.

Certain statistical analyses of the results were undertaken. These are summarized in Table XXII. The analyses consisted in comparing the controls with the benzol group to see whether significant differences existed. The data reveal that in all instances in which the results were tested the differences between the two groups can be considered significant. It should be borne in mind that in this comparison the entire benzol group, and not selected cases, was used.

SUMMARY AND CONCLUSIONS

1. Complete blood studies were performed on a relatively large group of individuals who had been exposed to concentrations of benzol vapor ranging from 11 to 1,060 p.p.m. for periods of from six to sixty months.

2. For comparison, similar studies were performed on a similar group of workers in a nonhazardous industry.

3. The abnormalities most frequently observed among the workers exposed to benzol were (a) anemia, (b) macrocytosis, and (c) thrombocytopenia.

4. Leucopenia was present in only a small percentage of the men who had been exposed to benzol.

5. Relative lymphocytosis was not found.

6. Comparison of the benzol group with the controls indicated that benzol exposure is likely to result in both relative and absolute lymphopenia.

7. Neutropenia was relatively rare; monocytosis, eosinophilia, and basophilia were not found in the benzol group.

8. Prolongation of the bleeding time following benzol exposure was rare in this study and did not parallel the reduction in thrombocytes.

9. Prolongation of the coagulation time was rare in the benzol group.

10. Relatively high hemoglobin values were a common finding in the benzol group. This abnormality was associated with macrocytosis as well as with overloading of red corpuscles with pigment.

11. The erythrocyte sedimentation rate is apparently not altered in benzol exposure.

12. The fragility of the erythrocytes does not seem to be significantly altered in benzol exposure but there seems to be a slight elevation of the serum bilirubin.

The studies covered in this paper were made while I was a member of the staff of the Division of Industrial Hygiene of the New York State Department of Labor. Through the courtesy of Dr. Leonard Greenburg, Executive Director of the Division of Industrial Hygiene, the data were made available for this report. Invaluable assistance in the statistical analyses was given by Dr. Morton Kramer, of the New York State Department of Health.

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THE ARTERIAL CIRCULATION OF THE LOWER EXTREMITIES IN CHRONIC ARTHRITIS*

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A STUDY of the arterial circulation of the lower extremities in chronic arthritis was suggested by the repeated observation of vascular disturbances in patients attending the Arthritis Clinic. Such circulatory abnormalities, particularly vasomotor, have been mentioned occasionally in treatises on arthritis. Search of the literature reveals little attention devoted to the arterial circulation of the limbs in the rheumatic diseases. The need for investigation in this field was pointed out recently in the annual survey of publications on arthritis.[1]

METHODS AND CRITERIA

The patients included in this series were suffering from arthritis and related conditions for which they were subjected to the routine physical and laboratory examinations of the clinic. They were then referred to the vascular department for study of the arterial circulation of their lower extremities. Every patient was examined by both of us. The diagnosis of arthritis or related condition was based on physical examination, laboratory data, such as total blood count, nonfilament count, sedimentation rate, uric acid content of the blood, and roentgen examination. The diagnostic designations were rheumatoid arthritis, active or inactive; typical or atypical. Other classifications according to symptoms and signs were osteoarthritis, combined arthritis (osteoarthritis and rheumatoid), fibrositis, gout, and sciatic syndrome.

The criteria for the diagnosis of arterial disturbance were, in the order of importance: plantar ischemia, relative coldness of the feet, reduced oscillometric readings at the pedal and ankle levels, and diminished amplitude or absence of palpable arterial pulsation. Roentgen evidence of calcified arteries of the legs and feet was also taken into consideration. Plantar ischemia was sought after elevation and repeated flexion of the feet, according to the method[2] described by one of us (S. S. S.). Rubor on lowering the extremities is not constant in incipient arterial disease but was noted when present. Relative coldness of the feet was estimated by a thermocouple and by palpation. Posterior tibial and dorsalis pedis pulses were palpated for their presence and amplitude. Pulse tracings of the feet and legs were made with a Tycos recording oscillometer.

It is necessary to distinguish arteriosclerosis from arteriosclerosis obliterans. The former denotes structural changes in the walls of the arteries not, however, necessarily causing obstruction to arterial onflow. Calcification may be present to such a degree as to be visible in roentgenograms, but there may not be sufficient narrowing or blockage of the lumen of the arteries to cause diminished

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flow. However, when the element of obstruction is introduced by narrowing of the lumen or by actual blocking with a thrombus, the terminology as well as the physical conditions are changed, resulting in arteriosclerosis obliterans.

Unilateral arterial spasm occurs in varying degree in incipient organic arterial disease. As a rule, spasm merely accentuates existing changes produced by the organic lesion. In other words, it may intensify ischemia, it may increase coldness and may further diminish the amplitude of arterial pulsation. Any or all of these effects of spasm may be either abolished completely or modified by injection of novocain about the posterior tibial nerve. If ischemia, temperature, and pulse amplitude are affected by this injection, one must still consider the underlying condition an organic lesion because of its unilateral occurrence. Pure spasm in an isolated extremity is rare except in the case of direct external trauma to the large arteries of the limb.

For these reasons, the presence of plantar ischemia, coldness, and diminished or absent oscillometric pulsation either alone or in combination, with asymmetric distribution, has been considered definite evidence of arterial disease. The cause of the arterial obstruction has been ascribed in every such instance to arteriosclerosis obliterans because of the common prevalence of this disease. Thromboangiitis obliterans is excluded because of its characteristic clinical picture of incidence in young males with accompanying venous involvement. The absence of superficial migrating phlebitis in any of these cases lends further doubt to the diagnosis of thromboangiitis obliterans as the cause of arterial obstruction in any of the cases here reported.

DIFFERENTIAL POSTERIOR TIBIAL BLOCK

In those patients presenting evidence of arterial obstruction, posterior tibial block, as described by Scott and Morton,[3] was performed. The procedure proved helpful in doubtful diagnostic situations. Anomalous response occurred in two patients, in one of whom the temperature rose without any accompanying increase in arterial pulsation; in the other an increased oscillometric reading was registered following the block, but the temperature rose only slightly. In addition to color and temperature observations, oscillometric pulse records were made before and after blocking.

FINDINGS

As Table I indicates, there were 47 patients with rheumatoid arthritis, 71 with osteoarthritis, nine with fibrositis, two with gout, two with gonorrheal arthritis, four with mixed arthritis, four with sciatica. The average age in the rheumatoid group was 44.4 years; in the osteoarthritis group, 51.3 years.

Plantar ischemia on elevation of the lower extremities, with or without brief flexion of the feet, was observed in 26 patients with rheumatoid arthritis, and in 22 patients with osteoarthritis. In a few patients with rheumatoid arthritis accompanied by vasomotor disturbances some plantar blanching was noticed in the normal position.

Palpable coldness, or lowered skin temperature, was noted in 26 rheumatoid patients and in 22 osteoarthritic patients. These observations confirm previous reports of the surface temperature changes in these diseases. The tem-

perature abnormalities were determined by thermocouple readings. It was found that gross estimation of temperature differences in the two extremities by the fingers of a trained observer were as reliable as instrumental readings in about 80 per cent of the cases.

TABLE I

TYPE OF ARTHRITIS	RHEUMATOID	OSTEOARTHRITIS	FIBROSITIS	GOUT	G.C.	MIXED	SCIATICA
Average age	44.4 Yr.	51.3 Yr.					
Paresthesia	5	5	0	1		1	11
Intermittent Claudication	2	1	0	1		0	
Coldness	28	28	1	1	1	2	2
Cyanosis	10	11	0	1		0	
Rubor	15	5	0	1		0	
Ischemia	24	23	1	1	1	4	2
Temperature changes	26	22	1	1		2	2
Pulsation absent	9	8	1	0		0	1
Oscillometer reading abnormal	22	18	1	2		0	2
X-rays	18	20	1	2		2	2
Total patients	47	71	9	2	2	4	4
Arteriosclerosis obliterans	3	2	1	1	0	0	1
Spasm	28 (59.5%)	23 (33%)	0	1	0	3	0
Negative	16	46	8	0	2	1	3
Arteriosclerosis obliterans %	6.3%	2.8%					
Total vascular disturbances	65.9%	35.2%					

Oscillometric readings were diminished in 22 patients with rheumatoid arthritis and in 18 with osteoarthritis. The amplitude of individual oscillations was important in determining any abnormal variation. Differences in readings at corresponding levels of both extremities, as illustrated in Figs. 1 and 2, were considered of great diagnostic importance.

Pulsations of one or more arteries were absent in nine patients with rheumatoid arthritis and in eight with osteoarthritis. Pulsations of the dorsalis pedis and of the posterior tibial arteries were studied. While an effort to determine the presence and the force of these pulses was made, this information was considered to have little, if any, diagnostic value in incipient arterial disease. Color changes on lowering the extremities were found in a small number of patients. Cyanosis of the feet was observed in ten patients with rheumatoid arthritis and in 11 with osteoarthritis. Rubor was noted in 15 patients with rheumatoid arthritis and in five with osteoarthritis.

Symptoms indicating intermittent claudication, despite the high incidence of circulatory abnormality, were elicited in only two patients with rheumatoid arthritis and in one with osteoarthritis.

Paresthesias of the lower extremities were described by five patients with rheumatoid arthritis and five with osteoarthritis. These symptoms are interesting and present a diagnostic problem in arthritis with arterial disturbance because of the difficulty of relegating them to either a circulatory or an arthritic basis.

In accordance with the above findings, as indicated in Table I, 28 patients with rheumatoid arthritis were considered as presenting evidence of vasospastic arterial disturbance; 23 patients with osteoarthritis showed signs of a similar condition. Three patients with rheumatoid arthritis gave conclusive evidence of arteriosclerosis obliterans, while two patients with osteoarthritis

appeared in the same category. When the findings were suggestive, but not definitely indicative of occlusion, the case was placed in the vasospastic group. There were eight such cases.

In a group of nine patients with fibrositis one showed signs of arteriosclerosis obliterans. In none of the others was there demonstrable evidence of arteriospasm. Of four patients with combined arthritis three had varying degrees of arteriospasm. One of four patients with sciatica showed arteriosclerosis obliterans; in three there were no vascular abnormalities. Of two patients with gout there were signs of arteriosclerosis obliterans in one and of arteriospasm in the other. Two patients with gonorrheal arthritis showed no circulatory disturbances.

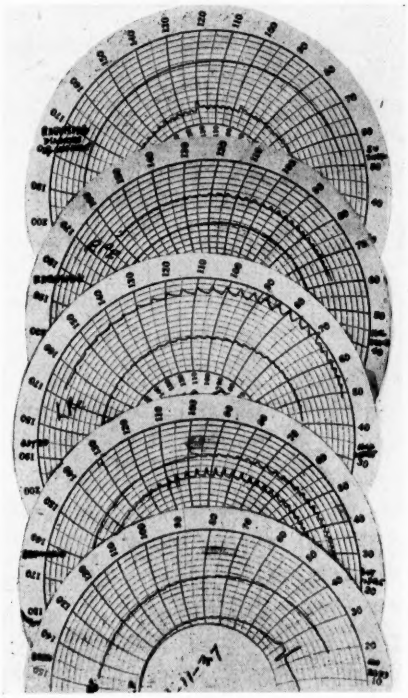

Fig. 1.

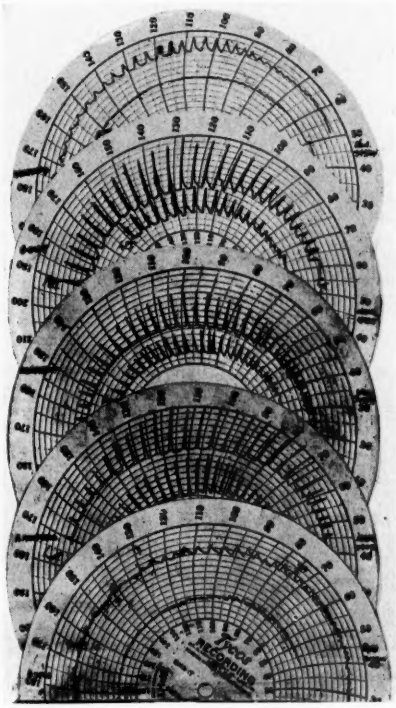

Fig. 2.

Fig. 1.—Reduced and unequal pedal pulses in arthritis. Each tracing shows both pedal pulses in the same patient.

Fig. 2.—Reduced and unequal tibial pulses in arthritis. Each tracing shows both tibial pulses in the same patient.

In our survey of 139 patients with rheumatic disease, therefore, seven presented signs of arteriosclerosis obliterans. Insufficient or no evidence for diagnosis of arterial disturbance occurred in 16 of 47 patients with rheumatoid arthritis, and in 47 of 71 patients with osteoarthritis.

In the series as a whole, of 47 patients with rheumatoid arthritis, 28, or 59.5 per cent, showed signs and symptoms of vasospastic arterial disturbance. Of 71 patients with osteoarthritis, 23, or 33.2 per cent, gave evidence of a similar condition. In the rheumatoid group three, or 6.3 per cent, gave indications of arteriosclerosis obliterans, whereas two, or 2.8 per cent, with osteoarthritis, presented a similar complication.

Within the rheumatoid group of 47 patients, then, 31, or 65.9 per cent, gave clinical evidence of abnormality in the arterial circulation of the lower extremities, while of 71 patients with osteoarthritis 25, or 35.2 per cent, presented such findings. Several patients in other classifications indicated in Table I showed signs of arterial disturbances, but these groups were so small that the individual cases, although suggestive, will not be considered further in this discussion. One patient with rheumatoid disease and another with osteoarthritis suffered from gangrene of an extremity. In each of these, however, diabetes mellitus was a pronounced complicating factor.

TABLE II

INCIDENCE OF INFECTIOUS ACTIVITY AND JOINTS INVOLVED IN ARTHRITIS WITH ARTERIAL DISTURBANCES

	TOTAL PATIENTS	PATIENTS WITH ARTERIAL SIGNS	SITE OF ARTHRITIC SIGNS AND SYMPTOMS WITH ARTERIAL DISTURBANCES				UNILATERAL ARTERIAL DISTURBANCES
			GENERALIZED	KNEES AND FEET ONLY	SPINE	UPPER EXTREMITIES ONLY	
Rheumatoid:							
Active	30	23	17	1		5	8
Inactive	17	8	6	1		1	1
Osteoarthritis	71	25	9	10	5	1	7

RELATION OF PERIPHERAL ARTERIAL DISTURBANCES TO LOCATION OF ARTHRITIS

A natural question arising in the course of this investigation was the possibility of a relationship between circulatory signs and arthritic involvement in the same extremity. As shown in Table II, a limited local reaction in isolated extremities did appear in a small number of patients and suggested as one possible source of vasomotor symptoms a local reflex angiospasm perhaps as a reaction to the nearby painful joint. While this study was confined to recording the arterial signs in the lower extremities, striking vasomotor symptoms in the upper limbs of these patients were frequently seen.

The influence of rheumatoid disease upon the location of vasomotor manifestations is indicated in Table II. Of 23 patients with active rheumatoid arthritis presenting vasomotor disturbances of the lower extremities, 17 had arthritic symptoms that involved all extremities, five showed limitation of joint trouble to the upper extremities, and only one to the knees and feet. In eight persons with inactive but symptomatic rheumatoid disease and associated circulatory signs in the lower extremities, six showed generalized joint involvement and discomfort; one presented arthritic symptoms restricted to the knees and feet, and one had involvement of the upper extremities only. These observations indicate that the location of peripheral vasospasm in many of the patients is unrelated to the site of rheumatoid activity.

Of 71 patients with osteoarthritis, as listed in Table II, 25 showed arterial disturbances of the lower extremities. Only ten of this group presented arthritic complaints limited to the joints of the lower extremities.

The higher, almost twofold, incidence of vasomotor symptoms found in rheumatoid disease as compared with osteoarthritis raises the question as to whether varied causative factors account for the difference. Close analysis

reveals that circulatory symptoms are not as prevalent in rheumatoid arthritis when the infectious process has subsided. Table II shows that the frequency of arteriospastic signs was highest in active rheumatoid arthritis (23 of 30 patients, or 77 per cent), next highest among those with inactive rheumatoid arthritis (8 of 17 patients, or 47 per cent), and least (25 of 71 patients, or 35.2 per cent) in osteoarthritis.

It is tempting to attribute the high incidence of peripheral arterial symptoms in active rheumatoid disease to the irritative effect of the underlying systemic infection. Such an assumption, however, could not clearly explain similar circulatory symptoms in apparently inactive rheumatoid arthritis and certainly does not account for vasospastic complaints in osteoarthritis which is generally considered to have a degenerative rather than an infectious basis.

It is readily discerned that the one clinical feature all of the three groups of arthritis present in common is symptomatic, painful articular disease. After thorough consideration of these facts one, therefore, must deduce that in these joint conditions some close, dynamic relationship may exist between painful, arthritic symptoms and the peripheral vasomotor imbalance with which they are frequently associated. Such a relationship appears most commonly, it is true, in the presence of active rheumatoid disease in which it is probable that systemic infection alone often may produce the circulatory disturbances by a local, toxic neurovascular reaction or, more likely, by central stimulation of the vegetative nervous system. It is further possible that systemic toxins in active, rheumatoid arthritis may supplement and augment the reflex mechanism initiating arterial disturbances, presumably as in quiescent rheumatoid infection and in osteoarthritis. This does not include the majority of patients with symptomatic osteoarthritis who do not present vasomotor disturbances.

TABLE III

JOINT X-RAYS OF 87 PATIENTS WITH VASCULAR DISEASE

SEX									
M	F	10-20	21-30	31-40	41-50	51-60	61-70	71-80	81-
70	17		1	6	22	28	26	4	

X-RAYS POSITIVE								HISTORY AND SIGNS POSITIVE	
RHEUMATOID ARTHRITIS		OSTEOARTHRITIS					NEGATIVE	POSITIVE	RHEUMATOID ARTHRITIS	OSTEOARTHRITIS
Negative	Positive	Knees	Foot	Sh	Sp	H				
	1	26	8	1	1	1	58 67.6%	28 32%	4 4%	15 17%

ARTHRITIS IN PATIENTS WITH VASCULAR DISEASE

As a control of the vascular study in arthritis, a group of patients with evidence of long-standing arterial disease attending the vascular clinic were studied for history, complaints, signs, and roentgen evidence of arthritis. According to Table III, of 86 individuals in this series under treatment for frank arterial disease, 58, or 67.6 per cent, gave no information or signs of rheumatic involvement at any time. Twenty-eight patients, or 32 per cent, yielded positive histories, physical signs, or roentgen evidence of rheumatic or arthritic involvement in some form.

THERAPY IN ARTERIAL DISTURBANCES IN ARTHRITIS

A variety of medical[4, 5] and surgical[6, 7] measures have been suggested to improve the peripheral circulation in arthritis. Although this report embraces an essentially diagnostic investigation, mention of some therapeutic experiences may not be irrelevant. A limited group of patients with arthritis showing vasospastic or occlusive disturbances were subjected to intravenous hypertonic saline therapy. Of 12 patients treated from three to eighteen months, seven showed marked improvement in symptoms, particularly in relief of discomfort in the lower extremities and in their ability to walk longer distances without the usual pain. The known angiospastic influence of tobacco warrants restriction of smoking in arthritis with severe vasomotor symptoms, together with other special care recommended for those patients with signs of arterial occlusion.[8] The accepted value of alcohol in circulatory disturbances makes it a worthy addition to therapy in arthritis[9] when vasospastic complaints are present.

SUMMARY AND CONCLUSIONS

1. In a clinical investigation of rheumatoid arthritis, 65.9 per cent of the patients showed abnormalities, usually vasomotor disturbances, of the arterial circulation of the lower extremities; in osteoarthritis 35.2 per cent of the cases presented similar findings. Three rheumatoid patients, or 6.3 per cent, had simultaneous arteriosclerotic occlusive disease; two with osteoarthritis, or 2.8 per cent, presented evidence of arteriosclerosis obliterans.

2. Study of those patients with arthritis presenting arterial abnormalities showed no direct, consistent relationship between the location of the vascular signs and the site of the arthritic process. The evidence suggests that the vasomotor disturbances in chronic rheumatoid arthritis and related conditions may be attributed to some systemic reaction probably acting through the sympathetic nervous system, although in some cases a local irritative reflex vasospastic mechanism in the painful arthritic extremity cannot be ruled out.

3. In a control group of 86 patients with advanced organic arterial disease, 28, or 32 per cent, offered a history, physical signs, or roentgen changes of rheumatic or arthritic involvement at some time. These findings approximate the incidence of such ailments in similar age groups of individuals without arterial disturbances and contribute clinical evidence that pronounced arterial disease alone does not usually produce arthritic signs and symptoms.

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EPINEPHRINE OUTPUT FROM THE ADRENAL GLANDS IN EXPERIMENTAL HYPERTHYROIDISM*

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IT IS often assumed that changes in irritability of the sympathetic nervous system that occur in hyperthyroidism are related to a corresponding increase in the rate of liberation of epinephrine from the adrenal glands. The investigation reported here was undertaken to obtain quantitative information on this question.

A significant role in the etiology and pathology of hyperthyroidism and of a number of other diseases, notably diabetes mellitus and arterial hypertension, is commonly attributed to the adrenal medulla. This is based chiefly on pharmacodynamic, sometimes toxic, reactions that are obtained on administration of epinephrine in pharmacologic doses. Support of quantitative measurements of the epinephrine output from the adrenals, under appropriate experimental conditions, is lacking.

Eppinger, Falta, and Rudinger[1] postulated a mutual stimulating interrelationship between the thyroid gland and the adrenal medulla. They suggested the probability that, in hyperthyroidism, the excess thyroid secretion may stimulate secretion of epinephrine from the adrenals, which in turn is responsible for increased irritability of the sympathetic nervous system. Such an interrelationship between the thyroid and adrenal glands seems unlikely, for, if it existed, a vicious cycle of mutually induced hypersecretion from both these glands might lead to accumulation of relatively large amounts of epinephrine and of thyroid hormone in the circulation.

We do not assume that experimentally created conditions are identical with similar conditions that occur clinically. Nevertheless, since certain of the pathologic manifestations often are attributed to an associated increase in the output of epinephrine from the adrenals, it seemed desirable to perform quantitative experiments for determination of the rate of liberation of epinephrine into the circulation in dogs subjected to prolonged thyroid feeding. These experiments are part of an investigation on possible functional interrelationships between the adrenals and the thyroparathyroid apparatus. Experiments related to the parathyroid will be reported in another paper.

Six adult dogs, three males and three females, received desiccated thyroid (Armour's), represented to contain 0.2 per cent iodine, for periods ranging from three to twelve and a half weeks. The dosage of thyroid ranged from

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0.5 Gm. to 1.3 Gm. per kilogram of body weight, administered daily together with the regular food. The routine diet consisted of a mixture of boiled beef lung, white bread, and bone meal.

Water intake and urine output were measured daily. Pulse rate and body weight were recorded at frequent intervals, and in four of the animals observations of the rectal temperature were made from time to time. In three dogs, one of which served as a control, observations of the basal metabolic rate were made as an indication of the degree of experimental hyperthyroidism that was induced.

Kunde[2] has demonstrated that thyroid feeding, in doses and for periods comparable with those of our experiments, induces a hyperthyroid condition in dogs. Our determinations of the basal metabolic rate were made essentially according to the method employed by Kunde. The dogs, previously trained to lie quietly, were fasted for eighteen to twenty hours. Each observation was preceded by a preliminary "rest" period. A specially devised mask was fitted to the animal and was connected with a Benedict-Roth closed circuit apparatus for determination of oxygen consumption. At the conclusion of each observation the rectal temperature and pulse rate were recorded. Observations of the basal metabolic rate were made approximately four times weekly throughout the course of the experiment.

After a sufficient number of observations were made to determine a base line for each factor, administration of thyroid was begun. At the end of the period of thyroid feeding the animals were sacrificed for determination of the rate of liberation of epinephrine from the adrenals. The epinephrine output was measured by the method of Stewart and Rogoff, obtaining adrenal vein blood at a known rate of flow, via the "cava pocket," and determining the epinephrine concentration in the blood by its action on rabbits' intestine segments. Ether anesthesia was employed for the operative procedure.

All the epinephrine assays were performed by the same individual (R. C.). Epinephrine output was determined in eleven normal dogs for comparison with the experimental animals, under the same laboratory conditions. These are included in Table I. The results obtained compare very well with those in the large series of animals reported by Stewart and Rogoff.[3]

Table II illustrates the results obtained in the experiments on thyroid-fed dogs. Definite loss in body weight was observed in all animals, the largest percentage loss having occurred in the two dogs (Nos. 19, 25) that were subjected to thyroid feeding for the longest periods. For convenience, the figures given in the table for heart rate, rectal temperature, and basal metabolic rate are the averages for the control period and for the period of thyroid feeding. It may appear from the averages that differences between the figures for observations before and during the period of thyroid feeding seem insignificant. However, the actual changes that occurred were generally large enough to leave no doubt about the genuineness of the results.

Thus, in dog 12, while the average temperature during the period of thyroid feeding does not appear to differ from that during the control period, actually it had reached 102.7° F. at the time that the animal was sacrificed for determination of the epinephrine output from the adrenals. A similar gradual increase

TABLE I

EPINEPHRINE OUTPUT IN CONTROL DOGS

RECORD NUMBER	SEX	BODY WEIGHT (KG.)	ADRENAL WEIGHT (GM.)		EPINEPHRINE OUTPUT PER MINUTE (MG.)	
			RIGHT	LEFT	PER DOG	PER KG.
A	M	7.9	0.49	0.49	0.00056	0.00007
B	M	8.1	---	---	0.00097	0.00012
C	M	5.0	---	---	0.00055	0.00011
D	M	11.5	0.62	0.75	0.0029	0.00025
E	F	9.7	0.95	1.02	0.00087	0.00009
F	M	12.9	0.55	0.53	0.00338	0.00026
G	M	8.6	0.51	0.51	0.00198	0.00023
H	F	6.5	0.51	0.51	0.0024	0.00037
I	M	8.9	0.60	0.51	0.0007	0.00008
J	M	8.3	0.45	0.56	0.00199	0.00024
K	M	8.2	0.39	0.40	0.00139	0.00017

TABLE II

EPINEPHRINE OUTPUT IN DOGS WITH EXPERIMENTAL HYPERTHYROIDISM

RECORD NUMBER	SEX	BODY WEIGHT (KG.)		THYROID FEEDING		AVERAGE HEART RATE PER MINUTE		AVERAGE BODY TEMPERATURE		AVERAGE B.M.R. CAL./KG./HR.		POLYURIA	EPINEPHRINE OUTPUT PER MINUTE (MG./KG.)
		INITIAL	FINAL	AVERAGE DAILY DOSE (GM./KG.)	DURATION (WEEKS)	BEFORE FEEDING PERIOD	DURING FEEDING PERIOD	BEFORE FEEDING PERIOD	DURING FEEDING PERIOD	BEFORE FEEDING PERIOD	DURING FEEDING PERIOD		
3	M	13.3	11.0	0.5	6	54	76					0	0.00016
6	F	5.1	4.5	1.0	3	66	96					+++	0.00014
10	F	10.7	9.8	1.3	6.5	84	88	101.8	102.2			+++	0.00013
12	M	9.9	8.6	1.1	5.5	70	76	101.6	101.7			+	0.00013
19	M	11.2	8.6	0.9	12.5	53	80	100.5	101.3	1.54	2.48	0	0.00013
25	F	7.3	5.7	1.1	12.5	71	83	100.3	101.4	1.71	2.54	++	0.00010

was observed in heart rate in all the animals. Water intake and urine output were increased in four of the animals. The two dogs (Nos. 19, 25) in which observations of the basal metabolic rate were made showed an average increase of 61 per cent and 49 per cent, respectively, during the period of thyroid feeding.

The rate of liberation of epinephrine from the adrenals, in the animals with experimental hyperthyroidism, was not significantly different from that of the control, normal dogs. It was well within the range of epinephrine output usually observed in normal animals when measured directly by this method. In a small number of preliminary experiments on dogs, subjected to thyroid feeding for shorter periods, one of us (J. M. R.) obtained similar results.

In four cats, studied by Mr. George N. Stewart, prolonged thyroid feeding led to marked loss of weight in two, moderate loss in one, and no loss in one. In all of the animals, at the end of the experimental period, he found the epinephrine output from the adrenals very close to the average for normal cats, as determined by the same method.

Our experiments indicate that thyroid feeding does not cause a significant change in the rate of liberation of epinephrine from the adrenals, even when it produces a definite hyperthyroid state in the experimental animal.

DISCUSSION

Recent experimental investigations support the concept of functional interrelationship between various endocrine organs. This is especially true concerning interdependence between the anterior hypophysis and certain other glands. Evidence in support of a hypothetical interrelationship of a mutual character between two glands is incomplete unless there is provision for maintenance of a physiologic balance between the interrelated functions. Existence of an interrelationship limited to mutual stimulation (or depression) alone, without a control mechanism to limit or prevent continued and increasing overactivity (in either direction) of the related organs, appears to us as unlikely.

In the case of thyroid-adrenal interrelationship, a mutual stimulating action is believed to exist. The literature, largely clinical, generally supports this view. The experimental evidence in support of this hypothesis, however, is susceptible to serious criticism: it is not quantitative; attempts to measure epinephrine in blood were made with inadequate methods; indirect, nonspecific reactions have been relied upon as indications of increased epinephrine output from the adrenals.

In blood serum of individuals with exophthalmic goiter, demonstrable increase in epinephrine was reported by Kraus and Friedenthal,[4] Fraenkel,[5] and Bröking and Trendelenberg[6] employing biological test objects. On the other hand, Gottlieb,[7] who tested plasma, was unable to obtain epinephrine reactions. O'Connor[8] demonstrated that the positive reactions were artifacts due to development of substances in clotting blood which gave reactions similar to epinephrine on the test objects.

Improper interpretation of such reactions may lead to serious error. Thus, Fraenkel,[5] obviously having employed rabbits' uterus segments without adequate control observations, concluded from the reactions obtained that serum

from systemic blood of normal individuals contains approximately 1:400,000 epinephrine, and that corresponding serum from patients with Basedow's disease contains four to eight times this amount. Arithmetical calculation indicates that he was misled by his tests. A concentration of 1:400,000 in the serum of systemic blood would correspond to approximately 1:2,000 in adrenal blood serum. Accordingly, in Basedow's disease concentrations corresponding to 1:500 to 1:250 adrenalin would be present in the adrenal vein blood serum, which seems highly improbable if not indeed impossible. The epinephrine concentration, reported by Fraenkel, for normal systemic blood is about 200 or more times as great as that found by Rogoff and Marcus[9] to be capable of effecting hemodynamic and other, sometimes toxic, reactions.

The tonus-increasing action on rabbit's uterus (and intestine) or on a vascular preparation (frog perfusion method) has been shown by Stewart[10] to be a serum effect, obtainable with unclotted blood, serum, or plasma. Epinephrine, however, produces inhibition of tone and contractions on rabbit's intestine, and the opposite on the nonpregnant or virgin uterus. This fact, indeed, incorporated in the method developed by Stewart and Rogoff, constitutes an important advantage over any other method at present available for quantitative assay of epinephrine in blood. It permits the elimination of artifacts produced by substances (or conditions) other than epinephrine, which yield epinephrine-like reactions on the intestine, uterus, vascular preparations, or denervated structures.

With sensitive test objects, Stewart[10] was unable to detect epinephrine in systemic blood obtained from a case of Grave's disease. Rogoff and Wasserman[11] obtained negative results in tests for epinephrine on systemic blood from patients with hypertension. Rogoff and Goldblatt[12] were unable to detect the presence of thyroid hormone in blood obtained from the thyroid vein during surgical operations for goiter; they employed the sensitive tadpole reaction in their tests.

Various attempts have been made to study the influence of thyroid extracts or thyroxin on the epinephrine output from the adrenal glands. None of these offer quantitative data. Ott and Scott[13] injected crude thyroid extracts and tested vena cava blood obtained through a catheter inserted through the femoral vein. Zunz and La Barre[14] performed crossed-circulation experiments in which intravenous injection of thyroxin was followed by determinations of the blood sugar level. They concluded that a gradual augmentation of epinephrine output occurs, which is associated with hyperinsulinemia caused by the thyroxin. Quantitative measurements of epinephrine output were not made.

Kuriyama[15] found no evidence that the acidosis induced in rabbits by thyroid feeding is induced by hypersecretion of epinephrine. Results obtained by histologic examination of the adrenals, e.g., Herring,[16] Cramer,[17] cannot be relied upon as an indication of the amount of epinephrine liberated by the glands. Like observations on the epinephrine load of the glands, they can indicate only the balance between production and liberation and do not indicate the output.

Gley and Quinquaud[18] obtained adrenal blood from the adrenal veins and studied its pressor effects when injected into the circulation of a test animal.

They found no significant difference between the effects of control specimens and specimens obtained after injection of thyroid extracts. Their experiments were the most direct but lack quantitative significance since they did not take account of possible changes in the rate of blood flow through the adrenals at the time of collection of the blood specimens.

In experiments where the observations were made shortly after administration of thyroid extract or thyroxin, it may be pointed out that thyroid effects could hardly have been expected. Kunde[2] demonstrated that changes in the basal metabolic rate do not occur until from seven to twelve hours after administration of thyroid. In our experiments thyroid feeding was continued for sufficiently long periods to cause definite thyroid reactions. Quantitative determinations of the rate of liberation of epinephrine from the adrenals were made with a method that has proved reliable over a period of many years.

Epinephrine output in the hyperthyroid animals was not different from that in normal, control dogs. If epinephrine secretion from the adrenal glands is at all related to the phenomena associated with hyperthyroidism, it appears more likely that under these conditions the sympathetic nervous system might become more sensitive to the action of epinephrine in the amounts normally liberated. This possibility is suggested by the observations of Asher and Flack[19] and others, and by the Goetsch[20] clinical test for hyperthyroidism.

SUMMARY

1. Experimental hyperthyroidism was produced in dogs by feeding desiccated thyroid for periods ranging from three weeks to three months. At the end of these periods the animals were sacrificed for determination of the rate of liberation of epinephrine from the adrenal glands.

2. Quantitative measurements of the epinephrine output from the adrenals showed that there is no detectable change in the rate of liberation of epinephrine. The output was within the same range as is found in normal, control animals.

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THE BLOOD SEDIMENTATION RATE IN HEALTHY GIRLS*

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SINCE the fundamental studies of Fåhraeus in 1917, the erythrocyte sedimentation test has become a very commonly used tool of the physician.[1] Despite the many possibilities of error in execution and interpretation, its comparative value has not diminished.

Abnormal sedimentation rates have been described in patients suffering from acute and chronic infections, malignancy, endocrine disturbances, infarction, and allergy. Pregnancy and meteorologic conditions seem to cause an increase in the velocity of sedimentation.[2] On the other hand, simple catarrhal inflammations, functional disorders, focal infections, and skin diseases do not seem to influence this test.

Most studies published have been concerned with the variations of the blood sedimentation rate in disease. The references listing physiologic changes in health describe a small variation between the two sexes, and an insignificant change in old age. A recent study of the rate in healthy young people disclosed a range of 1.0 to 30.0 mm. in 95 per cent of the cases, with a mean of 10.0 mm. in forty-five minutes. When plotted, a skew curve resulted, with the 15 mm. rate in forty-five minutes as the upper limit in 80 per cent of all the cases.[3] The Westergren pipette and the potassium oxalate method was used in this particular study.

In another examination of healthy men and women, weekly changes were found, but always within normal limits.[4]

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Normal limits for the blood sedimentation rate by the Westergren method are 1.0 to 3.0 mm. per hour for men, and 4.0 to 7.0 mm. per hour for women. The Cutler method assumes 2.0 to 10.0 mm. per hour as the average for women in health.[5, 6]

In a further attempt to obtain more accurate data concerning the blood sedimentation rates in healthy people, a total of 360 presumably healthy girls were studied with this idea in mind.

TABLE I

BLOOD SEDIMENTATION RATES OF 327 HEALTHY GIRLS

E.S.R. MM./HR.	NUMBER OF GIRLS IN AGE GROUPS (YEARS)											
	10	11	12	13	14	15	16	17	18	19	20	TOTAL
2	0	0	4	1	0	1	0	0	0	0	0	6
3	0	0	1	3	1	1	2	1	0	0	1	10
4	0	1	5	4	6	4	2	0	1	1	1	25
5	2	4	4	9	3	5	4	2	4	0	2	39
6	1	4	2	8	6	5	2	4	0	0	2	34
7	5	3	9	2	14	4	7	0	3	2	3	52
8	4	1	4	6	3	2	2	1	1	1	1	26
9	3	0	3	1	5	1	1	0	1	2	0	17
10	2	1	2	4	5	3	6	3	1	0	3	30
11	2	2	3	3	1	6	1	1	1	0	3	23
12	3	0	4	3	2	2	9	1	1	0	2	27
13	3	1	1	4	0	0	1	0	0	0	1	11
14	0	0	1	0	0	1	1	0	0	0	1	4
15	0	0	0	0	1	1	0	0	0	0	2	4
16	0	0	0	3	2	1	0	0	0	0	1	7
17	0	0	0	1	0	0	0	0	0	0	0	1
18	0	0	0	0	0	1	0	2	0	0	1	4
19	0	0	0	1	0	0	0	0	0	0	0	1
20	0	0	1	0	1	0	0	0	0	0	0	2
21	0	0	1	0	0	0	0	0	1	0	0	2
22	0	0	0	0	0	0	0	0	0	0	0	0
23	0	0	0	1	0	0	0	0	0	0	0	1
24	0	0	0	0	0	0	0	0	0	0	0	0
25	1	0	0	0	0	0	0	0	0	0	0	1

METHODS

Inasmuch as it has been shown that the erythrocyte sedimentation rate is reasonably the same by the methods of Linzenmeier, Westergren, and Cutler, the latter method, being available, was used.[7]

The 5 c.c. graduated tube was used, with 3 per cent sodium citrate as the anticoagulant. Necessary corrections were made for anemia. All the bloods were withdrawn between 10:00 and 11:00 A.M., and the tests were performed the same morning.

All the young women studied were between 10 and 20 years of age, and were born in the state of Vermont. Each girl was given the usual physical and laboratory examinations, which included routinely, serologic tests for syphilis and undulant fever, tuberculin test, chest x-ray film with serial retakes when indicated, and blood morphology examinations in all cases of increased sedimentation rates.

A definite attempt was made to rule out any hidden chronic infections as syphilis, tuberculosis, undulant fever, localized suppuration, and rheumatic fever. All these girls were followed for at least four months after the

determination of the original sedimentation rate to limit the possibility of any disease process appearing subsequently that may have been present at the time of the original examination.

No sedimentation rate was determined during a period of common cold, two days before or following menses, or when it was thought peripheral dental infection or tonsillitis might be present, despite previous statements that no appreciable elevation in rate is present in these conditions.[8]

RESULTS

There are included in this report the blood sedimentation rates of 327 presumably healthy girls. All these girls were known not to have been ill at any time within four months after the blood was withdrawn for the original test.

As can be seen from Table I, 92 per cent of these girls had sedimentation rates between the limits of 2 mm. and 13 mm. per hour, whereas the rates of 8 per cent of the girls ranged from 14 mm. to 25 mm. per hour. The median fell at 7 mm. per hour.

CONCLUSION

Normal variations of any test must be ascertained before such a test can be relied upon for diagnosis.

This study suggests that in young women the blood sedimentation rate is variable, and that about 10 per cent of healthy young women may have sedimentation rates of greater velocity than the average arbitrary range.

The diagnostic value, therefore, of the erythrocyte sedimentation rate must be guided by these normal variations.

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CLINICAL CHEMISTRY

STUDIES IN DYSTROPHIA MYOTONICA*

V. Creatine and Creatinine Excretion†

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AN ABNORMALITY in the metabolism of creatine and creatinine has been reported in several of the primary myopathies, as well as in certain conditions which secondarily affect the skeletal muscles. This abnormality may be manifested in several ways: (1) The creatine content of the muscles may be subnormal. (2) The ability to retain ingested creatine may be decreased. (3) Even on a creatine-free diet there commonly occurs a creatinuria which, according to Milhorat and Wolff,[9, 10] is an expression of the total mass of improperly functioning muscle rather than of the amount of muscle which has actually degenerated. (4) The excretion of creatine may be greatly augmented when glycine is administered orally. (5) If there are extensive muscular atrophy and degeneration, creatinine excretion, which is dependent upon the total muscle mass, may be considerably below normal. All these symptoms are observed to a marked degree in progressive muscular dystrophy.

The recent discovery at the Colorado General Hospital and Outpatient Clinic of a number of patients with dystrophia myotonica from five different families‡ has given an unusual opportunity for studies of this disease,[18-20, 22, 26] which is apparently not so rare as is commonly believed (cf. Stevenson in Cecil's *Textbook of Medicine*[24]). In the course of these studies it was decided to investigate the excretion of creatine and creatinine in some of the patients and to compare the findings with those in previous reports of this disease and of progressive muscular dystrophy.

The literature on the metabolism of creatine and creatinine in dystrophia myotonica is summarized in Table I. From this table the following observations may be made: (1) According to one investigator[15] creatine phosphate in resting muscles is below normal and its breakdown during contraction is subnormal. (2) Creatine tolerance is decreased in some cases. (3) Creatinuria is slight to moderate in degree and may or may not be augmented by glycine adminis-

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†The results given in this paper for patients J. M. and N. P. were taken from a thesis presented by Robert C. Lewis, Jr., to the Graduate School of the University of Colorado in partial fulfillment of the requirements for the degree Master of Science, August, 1937. A preliminary report of the work on patients J. M. and N. P. was made at the meeting of the American Association for the Advancement of Science at Denver, June, 1937.

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‡A patient from a sixth family has been admitted to the hospital since these studies were made.

TABLE I
SUMMARY OF REPORTS ON CREATINE AND CREATININE METABOLISM IN DYSTROPHIA MYOTONICA

TABLE I

SUMMARY OF REPORTS ON CREATINE AND CREATININE METABOLISM IN DYSTROPHIA MYOTONICA

AUTHOR	PATIENTS		CREATININE EXCRETION* (GM./24 HR.)	PREFORMED CREATININE COEFFICIENT*†	CREATINE EXCRETION (GM./24 HR.)	EFFECT OF GLYCINE ON CREATINE EXCRETION	CREATINE TOLERANCE‡	REMARKS
	NO.	SEX						
Pemberton[16]	1	F	0.270-0.574 (avg. 0.403)	—	—	—	—	
Bürger[2]	3	M	0.646-0.819	9.6-16.7	0.002-0.058	—	—	
Meyer[8]	2	?	—	Low	None	—	—	
Morgulis and Young[14]	1	M	0.717-0.725	—	0.353-0.562 (avg. 0.458)	—	Zero (ingested creatine recovered quantitatively as extra creatine and creatinine)	Creatine excretion increased by administration of meat protein; not affected by feeding 5.3 Gm. of gelatin per day
Kostakow and Slauck[7]	1	?	0.26-0.29	—	More than 0.02	No effect	—	
Slauck[23]	?	?	0.4 (approx.§)	—	0.03 (approx.§)	Glycine alone, no effect; glycine plus testicular extract, marked increase	Less than 5%	
D'Antona[4]	3	M	0.986-1.512‖	—	Slight to 0.158-0.298	Increase in 2 of 3 cases	—	
Adams, Power, and Boothby[1]	1	F	0.85	20.1	0.03	Slight increase	—	
Kolb, Harvey, and Whitehill[6]	2	M	—	—	Slight	—	Normal in one case; 50% in one case	Creatine excretion not affected by prostigmine or quinine
	1	F	—	—	Normal	—	Normal	
Milhorat and Wolff[13]	5	M	0.715-1.965	13.4-31.7	0.000-0.174	No effect or only slight increase	36-100%. Decreased in one case following glycine therapy	
	1	F	1.092	19.5	None	Caused slight excretion of creatine	75%. Decreased following glycine therapy	
Nevin[15]	?	?	—	—	—	—	—	Phosphagen in resting muscles below normal and its breakdown during contraction subnormal

*During control period.
†Milligrams of creatinine per kilogram per 24 hours.
‡Per cent of creatine retained.
§Values taken from graph of a representative case.
‖Creatinine excretion of patient who excreted largest amounts of creatine.

TABLE II

EXCRETION OF CREATINE AND CREATININE BY THREE PATIENTS WITH DYSTROPHIA MYOTONICA

PATIENT	PERIOD	DAYS	GLYCINE FED (GM./DAY)	NUMBER OF URINE SPECIMENS	TOTAL CREATININE (GM./DAY)	PREFORMED CREATININE (GM./DAY)	CREATINE (GM./DAY)	CREATININE COEFFICIENT*
J. M.	4/21 to 4/28	8	None	3	1.02	0.91	0.13	14.5
	4/29 to 5/ 2	4	5.4	3	1.05	0.92	0.15	14.4
	5/ 3 to 5/ 5	3	10.8	2	1.11	0.93	0.22	14.6
	5/ 6 to 5/16	11	30.0	2	1.39	1.01	0.44	
	5/17 to 5/20	4	15.0	2	1.12	0.85	0.31	
	5/21 to 8/ 5	76	30.0	27	1.35	0.99	0.42	14.9
	8/ 6 to 12/16	133	30.0	0				
	12/17 to 12/19	3	30.0	3	1.21	0.90	0.36	
	12/20 to 1/11	23	None	0				
	1/12 to 1/15	4	None	2	1.14	0.98	0.19	15.3
N. P.	6/17 to 7/22	35	None	26	1.08	1.03	0.06	13.9
	7/23 to 7/27	5	15	4	1.07	1.02	0.06	
	7/28 to 8/ 5	9	30	7	1.15	1.08	0.07	14.0
	8/ 6 to 8/21	16	30	0				
	8/22 to 12/25	126	10-30	0				
	12/26 to 1/ 9	15	30	0				
	1/10 to 1/13	4	30	3	1.26	1.17	0.11	
	1/14 to 1/27	14	None	14	1.15	1.05	0.11	14.0
R. B.	1/19 to 2/ 1	14	None	5	1.09	1.03	0.07	22.4†
	2/ 2 to 2/25	23	10-30	6	1.15	1.10	0.05	
	2/26 to 3/23	26	15-30 (anterior pituitary extract also given§)	2‖	1.28	1.23	0.05	
	3/24 to 4/ 7	15	None	0				
	4/ 9 to 4/24	16	15-30 (testosterone propionate also given¶)	3‖	1.22	1.21	0.01	

*Milligrams of creatinine per kilogram per 24 hours.

†Based on weight of patient on Jan. 8.

‡Values the same in middle of period as at end of period.

§A total of 20 c.c. of anterior pituitary extract (Squibb) was administered in twelve injections (three times a week).

‖Specimens collected at end of period.

¶A total of 135 mg. of oretan was administered in twelve injections.

tration. According to Milhorat and Wolff,[13] the excretion of creatine is much less than in patients with progressive muscular dystrophy who show similar degrees of muscular disability. (4) Creatinine excretion is variably decreased.

EXPERIMENTAL

The excretion of creatine and creatinine was studied in one woman (J. M.) and two men (N. P. and R. B.) with dystrophia myotonica. J. M., female, aged 44 years, had severe myotonia in the hand grasps, and moderate atrophy of the forearms, hands, and lower extremities. She was still menstruating although somewhat irregularly. Her basal metabolic rate was minus 10. N. P., male, aged 52 years, had slight myotonia, marked atrophy of the forearms and hands, and

moderate atrophy of the lower extremities, but no evidence of testicular atrophy. His basal metabolic rate was minus 20. R. B., male, aged 27 years, had moderate myotonia, moderate atrophy of the forearms and hands and slight atrophy of the lower extremities. His testicles were small and questionably atrophic. His basal metabolic rate was minus 30. A more detailed description of these patients may be found in an earlier paper.[26]

Determinations of creatine and creatinine in twenty-four-hour urines (7:00 A.M. to 7:00 A.M.) were made during a preglycine period, during glycine administration, and after discontinuing glycine. The amino acid was administered by mouth three times daily after meals in amounts as high as 30 Gm. per day. No attempt was made in any case to control the diet.

Preformed creatinine was determined by the Folin colorimetric method[5] using a Duboscq colorimeter and matching through a piece of cobalt glass. Total creatinine was determined by the same method after the urine had been autoclaved with picric acid according to the method of Folin.[5] Creatine was calculated by multiplying the difference in the above determinations by the factor 1.159.

Average values for the different experimental periods on these three patients are shown in Table II.

The woman patient, J. M., showed a creatinuria which was significantly higher than the average daily excretion of the normal women studied by Rose.[21] In this patient creatine excretion increased definitely during glycine administration; the increase was prompt and the excretion remained high as long as glycine was fed, a period of eight months. The amount of creatine in the urine depended apparently on the amount of glycine fed, for it increased progressively as the dosage of glycine was raised and fell when the dosage of glycine was lowered. Three weeks after cessation of glycine administration the creatinuria had returned almost to the preglycine level.

Both of the men patients showed a slight initial creatinuria. The amounts of creatine excreted were approximately the same as those reported in patients with dystrophia myotonica by most other investigators, but these values are not as great as some of those reported for normal men by Taylor and Chew[25] and by Milhorat and Wolff.[11] In neither man was there any immediate effect on creatine excretion when glycine was fed; in patient R. B. there was no effect at any time. However, after glycine had been fed to patient N. P. for five and one-half months, the creatinuria had increased from the preglycine level of 0,06 Gm. per day to 0.11 Gm. per day, and it was still at this higher level two weeks after cessation of glycine feeding. Such a small increment in creatine excretion may have little significance, however, since Braestrup[3] found that 14 Gm. of glycine daily produced a maximum excretion of 0.080 Gm., 0.094 Gm., and 0.100 Gm. per day in three normal men who had shown no initial creatinuria. On the other hand, Braestrup[3] found that glycine produced no creatinuria in another normal man, and Milhorat and Wolff[11] reported that glycine feeding did not increase the creatine excretion of normal men who showed a preglycine creatinuria comparable to that of our two men patients. These reports were all concerned with relatively short periods of glycine feeding; no reports were found that showed

the effect on creatine excretion of administering glycine to normal men for such a prolonged period and in such large amounts as in our experiments.

Patient R. B., whose creatinuria was not augmented by glycine alone, had no greater excretion of creatine when anterior pituitary extract or testosterone propionate* was administered simultaneously with glycine. These results differ from those of Slauck[23] in a patient with dystrophia myotonica but are in accord with those of Milhorat and Wolff[12] in patients with myasthenia gravis. Slauck found that his patient, who showed no increase of creatine excretion in response to glycine alone, had a markedly increased creatinuria when testicular extract was administered simultaneously with glycine. Milhorat and Wolff reported that the administration of anterior pituitary extract to two patients with myasthenia gravis had no effect on creatine excretion. It is interesting to observe that Pizzolato and Beard[17] have reported an increase in the creatine content of muscle and in the excretion of creatine when anterior pituitary extract is administered to normal rats, and also an increase in the creatine content of muscle when testicular extract is given.

Creatinine excretion in all three patients fluctuated considerably throughout the experiments and was not significantly affected by glycine administration. Part of the variation may be ascribed to changes in diet from day to day. The creatinine coefficient was within the normal range in two of the patients but was low in patient N. P., who showed the greatest degree of muscle atrophy.

SUMMARY AND CONCLUSIONS

1. The excretion of creatine and creatinine and the effect of glycine administration thereon were studied in three patients, one woman and two men, with dystrophia myotonica.

2. Creatinuria was present in all three patients during a preglycine period. The amounts of creatine excreted by the woman were greater than normal; the amounts excreted by the two men were small and probably within normal limits.

3. Glycine administration produced an increase in the amounts of creatine excreted by the woman and by one of the men. In the woman a definite increase occurred promptly; in the man the rather slight increase was slow to develop and of questionable significance. Neither the administration of glycine alone, of glycine and anterior pituitary extract, nor of glycine and testosterone propionate affected the excretion of creatine in the other man.

4. The excretion of creatinine was subnormal in the patient who showed the greatest degree of muscle atrophy but was normal in the other two patients. It was not significantly affected by glycine administration in any of the three patients.

5. Although the data obtained are insufficient for drawing definite conclusions, they seem to augment the evidence from previous reports that the slight to moderate creatinuria which may be observed in dystrophia myotonica is neither as constant nor as striking a finding as that observed in progressive muscular dystrophy, and is less strongly affected by administration of glycine than in the latter disease.

*The testosterone propionate (oretan) was furnished through the kindness of Dr. Max Gilbert of Shering Corporation.

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BIOCHEMICAL STUDIES OF THE BLOOD OF DOGS WITH N-PROPYL DISULFIDE ANEMIA*

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PREVIOUS studies by this laboratory of pernicious anemia[1] and the hemolytic and hypochromic anemias of childhood[2] have shown that chemical changes occur in the blood which are indicative of abnormal erythrocyte composition or structure. Further study and elucidation of the chemical structure of the normal erythrocyte has been made possible by the development of a method[3] for the preparation of posthemolytic residue or stroma practically free of hemoglobin. Chemical studies of this material have revealed that the stroma of the normal erythrocyte is a protein-lipid complex of similar composition for various mammalian species.[4, 5] Moreover, it has been demonstrated that the lipid distribution in the posthemolytic residue is similar to that in the intact erythrocyte.[4]

In view of the indications of abnormal erythrocyte structure in certain anemias, it seemed desirable to secure sufficient stroma for a complete chemical investigation of its character, together with hematologic and chemical observations on the intact erythrocyte. At present enough blood from human patients with anemias for preparation of sufficient stroma is practically impossible to secure. It is possible, however, to develop an experimental anemia in animals, a procedure which offers the advantages of more rigid control, but has disadvantages in that the particular types of anemia occurring in human beings which present the most striking changes in erythrocyte structure[1, 2] are not amenable to experimental development. Simple hypochromic anemia (iron deficiency), which results in impaired hemoglobin formation, does not appear to affect the chemical design of the erythrocyte.[2] Hemolytic anemias, which are accompanied by definite alterations in the hematologic pattern of the blood, are produced in animals by certain drugs causing red blood cell destruction. Such an anemia offers an excellent opportunity for correlated hematologic, chemical, and stroma studies which are important in view of the marked abnormalities of erythrocyte composition found in the congenital hemolytic anemias of childhood.[2]

Sebrell[6] originally observed that onions would produce a severe anemia in dogs. Gruhzit[7, 8] confirmed this and showed further that the action resided in

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the onion oil, probably due to the allyl propyl disulfide, the main constituent of onion oil. In a study of different disulfide compounds Gruhzit[8] demonstrated that N-propyl disulfide produced a severe anemia in dogs and that any desired degree of anemia can be maintained for a long period of time, depending on the regular administration of definite amounts of the disulfide, a significant factor in studying the influence of anemia on erythrocyte structure.

Gruhzit[8] has described the changes in the hematologic picture induced by this type of anemia, but thus far no chemical observations have been reported. Furthermore, the possibility of using N-propyl disulfide to control polycythemia in human beings[9] points to the necessity of studying not only the hematologic and chemical changes occurring in the blood but also the pathologic changes in the tissues as well. This report includes, therefore, detailed chemical and hematologic examinations of the blood and pathologic observations of certain tissues, in addition to the effect of anemia on the composition of erythrocyte stroma.

EXPERIMENTAL

Two originally healthy dogs were maintained in a moderate-to-severe degree of anemia for a period of over three months by the administration of N-propyl disulfide ($CH_3CH_2CH_2S$-S $CH_2CH_2CH_3$).* Previous to beginning the study, the animals were immunized with *B. bronchisepticus* vaccine. The dogs were fed a complete, nutritive diet and given the disulfide in gelatin capsules at intervals and dosages, as indicated in Chart 1. Hematologic examinations consisting of hemoglobin determinations, red blood cell and white blood cell counts were made at intervals during the course of the experiment. These values, plotted in Chart 1, show the degree and maintenance of the anemias as the study progressed. Samples of blood (60 c.c.) for chemical analysis were taken at the beginning of the experiment before disulfide was administered, frequently in the first month during the period of adjustment, and at the end of the study, as indicated in Chart 1. Determinations of red blood cell count, hemoglobin content, hematocrit value, red blood cell diameter, specific gravity, and water content were made; from these, corpuscular volume, weight, thickness, area, hemoglobin, and water were calculated. The chemical determinations on the blood included the following: serum calcium and inorganic phosphorus, total protein of the plasma; sodium, potassium, and chloride content of serum and cells and the complete lipid distribution of the plasma and erythrocytes. The methods used were the same as those in previous studies reported from this laboratory.[10, 11]

Just preceding completion of the experiment, x-rays† of the skull, spine, and leg bones of both dogs were made. When the experiment was terminated, the dogs were exsanguinated under light ether anesthesia and the blood was used for the preparation of erythrocyte stroma.[3] Tissues were preserved for histopathologic and chemical study.

*This study was made possible through the courtesy and facilities of Parke, Davis & Co., Detroit. E. A. Sharp, M.D., medical director, suggested the use of N-propyl disulfide to produce the anemia and gave helpful suggestions during the course of the investigation. L. T. Clark, Ph.D., director of the biological laboratories, made the animals available and the investigation possible. O. M. Gruhzit, M.D., pathologist, prepared the anemic animals, made the hematologic examinations on the blood, took the blood and tissue samples, and gave helpful advice in the preparation of the manuscript.

†The x-ray films were taken and read by Lawrence Reynolds, M.D., roentgenologist, Harper Hospital, Detroit.

TABLE I

HEMATOLOGIC OBSERVATIONS ON DOGS WITH DISULFIDE ANEMIA

DOG	HEMATOCRIT VALUES (%)	RED BLOOD CELLS		HEMOGLOBIN		CORPUSCULAR MEASUREMENTS					WATER PER CELL ($\mu\mu$g)
		WHOLE BLOOD (MILLIONS/C.MM.)	PER GRAM CELLS ($\times 10^{10}$)	WHOLE BLOOD (GM./100 C.C.)	PER CELL ($\mu\mu$g)	VOLUME (C. μ)	WEIGHT ($\mu\mu$g)	DIAMETER (μ)	THICKNESS (μ)	AREA (SQ. μ)	
Normal:*											
Average	51	6.4	1.15	13.8	22	80	87	6.8	2.3	120	55
Range	48-53	5.8-7.3	1.11-1.26	12.5-15.0	19-26	73-83	80-90	6.6-6.9	2.2-2.4	118-123	52-61
146:											
Pre-experimental	43	6.5	1.38	11.7	18	66	72	–	–	–	–
After induction of anemia:											
Within first month:											
Average†	25	3.4	1.19	6.3	19	77	86	7.5	1.8	129	57
Range	18-33	2.0-4.6	0.99-1.31	3.4-8.8	13-23	71-93	76-101	7.4-7.5	1.6-2.1	126-138	53-67
After 3 months	30	3.3	1.00	8.2	25	93	100	7.4	2.2	137	68
148:											
Pre-experimental	44	7.1	–	12.9	–	–	–	–	–	–	–
After induction of anemia:											
Within first month:											
Average‡	23	3.0	1.20	5.3	18	80	87	7.4	1.9	130	60
Range	18-29	1.7-4.2	0.86-1.33	3.9-7.3	14-27	70-108	75-116	7.3-7.6	1.6-2.4	123-148	52-76
After 3 months	32	3.5	1.03	7.8	22	90	98	7.5	2.0	135	66

*Five determinations on 3 healthy dogs.
†Average for 4 samples.
‡Average for 5 samples.

TABLE II

DISTRIBUTION OF WATER, PROTEIN, AND MINERALS IN BLOOD OF DOGS WITH DISULFIDE ANEMIA

DOG	RED BLOOD COUNT (MILLIONS/C.MM.)	SERUM INORGANIC PHOSPHORUS (MG./100 C.C.)	SERUM CALCIUM (MG./100 C.C.)	PLASMA PROTEIN (GM./100 C.C.)	SERUM				ERYTHROCYTES				CORPUSCLE		
					SODIUM (MEQ./LITER)	POTASSIUM (MEQ./LITER)	CHLORIDE (MEQ./LITER)	WATER (GM./LITER)	SODIUM (MEQ./LITER)	POTASSIUM (MEQ./LITER)	CHLORIDE (MEQ./LITER)	WATER (GM./LITER)	SODIUM (MEQ. × 10^{-13})	POTASSIUM (MEQ. × 10^{-13})	CHLORIDE (MEQ. × 10^{-13})
Normal:*															
Average	6.4	4.2	12	6.2	148	8	110	944	118	7	52	717	94	6	41
Range	5.8-7.3	3.0-5.3	11-13	5.9-6.6	140-152	5-11	107-112	942-946	114-128	4-13	41-58	701-732	91-96	3-11	30-46
146:															
Pre-experimental	6.5	4.8	12	6.7	143	–	109	939	109	–	52	724	72	–	35
After induction of anemia:															
Within first month:															
Average†	3.4	5.4	12	6.8	148	15	116	938	100	7	58	732	77	5	46
Range	2.0-4.6	5.1-5.9	11-12	6.2-7.4	147-149	6-17	112-120	935-940	78-121	3-12	47-78	718-738	73-88	2-8	36-57
After 3 months	3.2	4.3	12	6.2	150	6	96	944	106	21	25	736	99	20	23
148:															
Pre-experimental	7.1	3.9	12	7.2	146	–	105	–	110	–	53	–	88	–	42
After induction of anemia:															
Within first month:															
Average‡	3.0	5.1	11	6.7	145	8	112	938	120	25	66	741	94	18	51
Range	1.7-4.2	4.4-5.5	10-12	6.3-7.6	138-150	5-10	104-115	932-942	103-165	6-37	56-82	735-749	75-115	6-26	45-60
After 3 months	3.5	4.7	–	6.5	146	5	93	937	115	19	28	734	104	17	26

*Four determinations on 3 healthy dogs.
†Average of 4 samples.
‡Average of 5 samples.

RESULTS

The clinical blood picture during the course of the anemia is given in Chart 1 and demonstrates the marked anemia producing powers of N-propyl disulfide and, furthermore, shows the closely parallel responses of the two dogs to the drug. Table I presents the hematologic observations on the blood samples taken for chemical analysis. Before administration of the disulfide the two dogs had blood counts of 6.5 and 7.1 millions per cubic millimeter, respectively. During the first month of the study these values ranged between 1.7 and 4.6 millions, after which the level approximated 3.5 millions for both animals until the termination

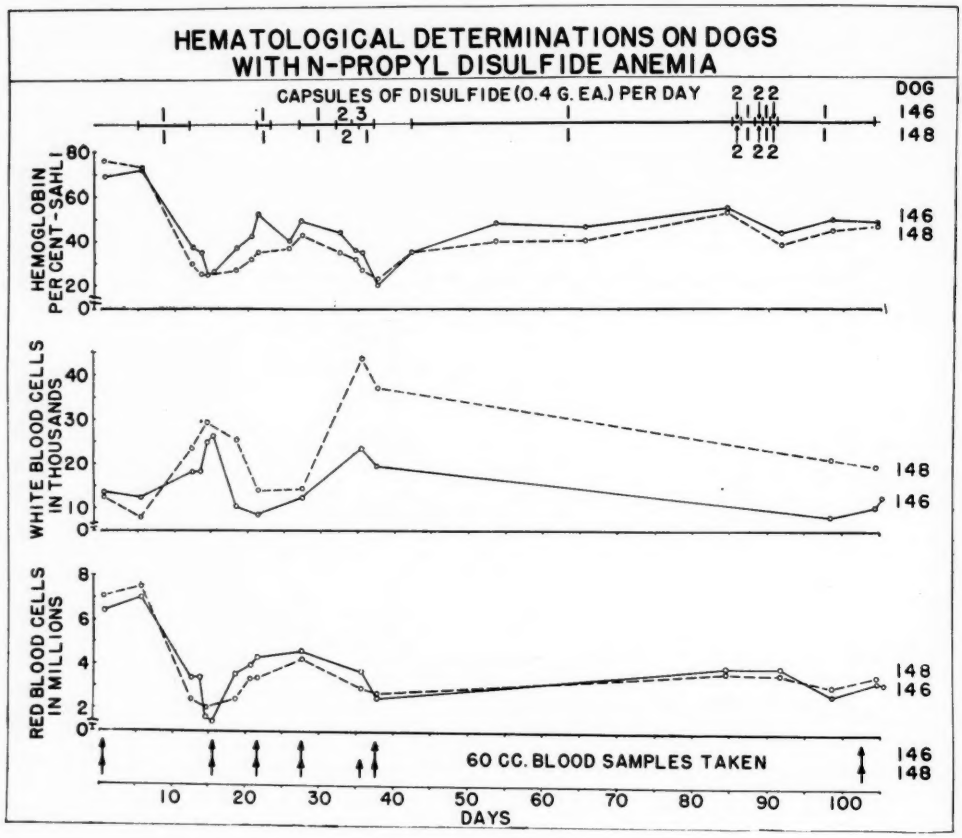

Chart 1.

of the experiment. Specific gravity, water, and protein contents of the plasma showed no change as a result of the anemia (Table II), which indicates that the blood concentration was unaffected. All corpuscular measurements disclosed a larger and heavier cell in the anemic state. The cell volume was about 80 c.μ in the animals before anemia was induced, but with the progress of the anemic condition the cell volume increased to 90 c.μ or over. Likewise, the corpuscular weight was 90 micromicrograms or less in the nonanemic bloods, but increased to a weight of 98 micromicrograms or more in the anemic condition. Similarly, the cell diameter and the calculated cell area were increased with the anemia, whereas the computed number of cells per gram of cells was decreased. Although part of

the increased weight may have been due to a larger quota of hemoglobin per cell, it is accounted for chiefly by an augmented water content. Whereas the corpuscular water of the normal dog erythrocytes averaged 55 micromicrograms, it rose as high as 76 micromicrograms during the anemia, changes accompanied are shown by a corresponding decrease in specific gravity. These alterations indicate that the cells underwent osmotic swelling, which is in accord with their increased fragility in hypotonic sodium chloride solutions. Hemolysis was evident in 0.62 per cent sodium chloride (the highest concentration used) in comparison with normal values of 0.50 to 0.54 per cent given by Haden.[12]

The sodium, potassium, and chloride distribution of the blood serum and cells, together with the serum calcium and inorganic phosphorus values, are given in Table II. The plasma mineral constituents, except the chloride, appear to be unaffected by the induction of the anemia. The chloride content of both the serum and erythrocytes was consistently lower after three months of anemia. Serum chloride was above 100 meq. per liter in the animals without anemia, while at the end of the study it was 96 meq. or less. Similarly, the chloride content of the erythrocytes was reduced markedly, from above 50 meq. in the animals when nonanemic to below 30 meq. after three months' duration of the anemia.

TABLE III

DISTRIBUTION OF MINERALS IN WATER OF PLASMA AND ERYTHROCYTES

(Meq. per liter of water)

DOG	PLASMA				ERYTHROCYTES			
	SODIUM	POTASSIUM	TOTAL BASE	CHLORIDE	SODIUM	POTASSIUM	TOTAL BASE	CHLORIDE
Normal:*	157	9	163	117	164	10	174	73
146:								
Pre-experimental	152	–	–	116	151	–	–	72
Within first month†	157	16	173	124	137	10	147	78
After 3 months	159	6	165	102	144	29	175	34
148:								
Within first month‡	155	9	164	112	162	34	196	89
After 3 months	155	5	160	100	156	27	183	38

*Four determinations on 3 healthy dogs.
†Average of 4 samples.
‡Average of 5 samples.

The advantages of expressing the erythrocyte composition in terms of an individual corpuscle rather than on the unit weight basis has been demonstrated in the anemias.[1, 2] In this study, calculation of the corpuscular mineral concentration emphasizes further the lowered chloride content of the erythrocyte at the termination of the experiment (Table II). In addition, there is evidence of an elevated base (sodium and potassium) content per cell which is not particularly apparent from the values expressed in milliequivalents per liter of erythrocytes. Before anemia the dogs had 72 and 88 $\times 10^{-13}$ meq. of sodium per cell, whereas after three months in the anemic state, there were 99 and 104 $\times 10^{-13}$ meq. of sodium, respectively, in comparison with normal values of 91 to 96 $\times 10^{-13}$ meq. Likewise, potassium was elevated after duration of the anemia to 17 and 20 $\times 10^{-13}$ meq. of potassium in contrast to a normal value of 3 to 6 $\times 10^{-13}$ meq. per

TABLE

DISTRIBUTION OF BLOOD LIPIDS IN

DOG	RED BLOOD COUNT (MILLIONS/C.MM.)	PLASMA (MG./100 C.C.)							ERYTHRO-	
		TOTAL LIPID	NEUTRAL FAT	FREE CHOLESTEROL	CHOLESTEROL ESTERS	PHOSPHOLIPID			TOTAL LIPID	NEUTRAL FAT
						TOTAL	CEPHALIN	CHOLINE§		
Normal:*										
Average	6.4	632	137	31	156	308	82	226	615	99
Range	5.8-7.3	583-680	130-148	19-43	144-174	268-357	44-153	204-245	448-699	0-190
146:										
Pre-experimental	6.5	566	69	37	174	286	69	217	727	183
After induction of anemia:										
Within first month:										
Average†	3.4	565	150	41	154	221	49	172	1035	283
Range	2.0-4.6	488-674	82-203	38-43	132-194	171-277	21-72	138-205	487-1408	23-471
After 3 months	3.2	412	173	33	70	136	0	136	793	150
148:										
Pre-experimental	7.1	446	22	35	119	270	108	162	934	374
After induction of anemia:										
Within first month:										
Average‡	3.0	576	143	40	148	244	69	175	931	192
Range	1.7-4.2	459-739	70-183	31-59	115-211	143-338	40-104	103-260	682-1407	0-490
After 3 months	3.5	419	161	28	77	153	0	153	881	313

*Five determinations on 3 healthy dogs.
†Average of 4 samples.
‡Average of 5 samples.
§Lecithin plus sphingomyelin.
**Represents the concentration of an average single red blood cell.

cell. The elevated corpuscular base, together with the lowered chloride, indicates an excess cation content or the presence of increased amounts of an undetermined anion, alterations which are similar to those noted in various types of human anemias.[1, 2, 13]

The anemia was accompanied by changes in the erythrocytes indicative of osmotic swelling. Calculation of the mineral composition on the basis of milliequivalents per liter of cellular and plasma water (Table III) bring out the fact that normally, as well as in the anemia, the sodium is almost evenly distributed between the water of plasma and cells. Normally, the potassium is likewise equally partitioned, but as the anemia progresses it becomes more concentrated in the cells. On the other hand, although the chloride is lowered in the water of both plasma and cells, it is more markedly diminished in the cells, which is in agreement with the swelling of the cell and its increased fragility.

The lipid composition of the plasma and erythrocytes (mg. per 100 c.c.), together with the calculated corpuscular concentration (mg. $\times 10^{-12}$ per cell), are given in Table IV. The total lipid content of the plasma of the dogs before

IV

DOGS WITH DISULFIDE ANEMIA

CYTES (MG./100 C.C.)					CORPUSCLE (MG. × 10^{-12})**						
FREE CHOLESTEROL	CHOLESTEROL ESTERS	PHOSPHOLIPID			TOTAL LIPID	NEUTRAL FAT	FREE CHOLESTEROL	CHOLESTEROL ESTERS	PHOSPHOLIPID		
		TOTAL	CEPHALIN	CHOLINE§					TOTAL	CEPHALIN	CHOLINE§
132	15	369	187	182	492	81	104	12	295	149	146
104-148	0-35	271-467	150-240	99-255	327-574	0-156	85-118	0-26	198-386	123-198	72-209
76	26	442	274	168	483	122	50	17	293	182	112
159	0	593	352	241	820	221	123	0	476	272	203
147-171	0-0	293-916	293-444	0-568	353-1311	17-335	105-154	0-0	212-853	212-324	0-529
162	130	351	112	239	738	140	151	121	326	104	222
132	56	372	190	182	747	299	106	45	298	152	146
157	40	542	255	287	726	140	123	30	432		249
102-229	0-98	350-893	0-493	0-596	504-979	0-362	75-159	0-68	264-642	183	0-642
161	17	390	74	316	794	282	145	15	352	0-364	285
										67	

anemia ranged between 446 and 680 mg. At the end of the study the experimental animals had a plasma lipid content of 412 and 419 mg. Of the various components of the total lipid, free cholesterol appears to have been unaffected by the induction of the anemia; on the other hand, neutral fat was increased and cholesterol esters and phospholipid were decreased. Although dogs 146 and 148 had a lower plasma neutral fat, 69 and 22 mg., respectively, before induction of the anemia as compared to an average of 137 mg. for the animals that received no drug, they had concentrations of 173 and 161 mg. at the termination of the experiment. The cholesterol ester concentration of the plasma ranged between 119 and 174 mg. in the nonanemic animals. After three months of N-propyl disulfide the anemic dogs (146 and 148) had a plasma content of 70 and 77 mg. of cholesterol esters, respectively. Similarly, the plasma phospholipids ranged between 270 and 357 mg. in the animals when healthy, and fell after three months of anemia to 136 mg. in dog 146 and 153 mg. in dog 148. Separation of the phospholipid fraction into cephalin and choline phospholipid (lecithin plus sphingomyelin) showed that, whereas both follow a trend similar to the total phospholipid, the cephalin appears to be affected to a greater and more consistent degree. In fact, no cephalin could be detected in the plasma of the dogs after being kept anemic for three months with disulfide. These parallel changes in the plasma

lipids of the two dogs—lowered phospholipid and cholesterol esters and increased neutral fat—are typical of the changes found in the plasma lipids of different types of anemias in man and animals.[1, 2, 14]

A study of erythrocyte lipids (on the basis of milligrams per 100 c.c. of cells) indicates that although the total lipid concentrations in the two animals varied widely during the first month of the anemia, the values at the end of the experiment appear comparable to those obtained before N-propyl disulfide was given. The neutral fat concentration shows the widest variation of any of the lipid components, but without relation to the anemia. Free cholesterol seems to have been increased, and the total phospholipid appears to have undergone no consistent change as the result of the anemia.

Due to the changing size and weight of the red blood cells in anemia, however, determinations on the unit weight basis may obscure what is actually occurring in the individual corpuscle. Calculation of the composition of the individual cell, therefore, gives a truer picture of the chemical changes in structure, well illustrated by the present study. Both dogs exhibited an increased corpuscular concentration of total lipid not evident from the data on total lipid per 100 c.c. of erythrocytes. Neutral fat data show no change attributable to the anemia. The increase in free cholesterol is brought out more strikingly in the corpuscular concentration. The animals which received no disulfide had a range of 85 to 118 mg. x 10^{-12} per cell, and the experimental dogs had values of 50 and 106 x 10^{-12} before administration of the drug. At the end of the three months of anemia there was an increase to 151 and 145 mg. x 10^{-12} per cell, respectively.

The rise in cholesterol ester content of the corpuscle, noted in previous studies of anemia,[1, 2] was observed in dog 146; the cholesterol esters increased from 17 x 10^{-12} mg. per cell to 121 x 10^{-12} mg. at the end of the experiment. The other experimental animal (dog 148) showed little or no change in the corpuscular content of cholesterol esters.

The concentration of the corpuscular phospholipid was increased in both dogs as a result of the anemia, in contrast to the observations per liter of cells. The average concentration of phospholipid per single cell was 295 x 10^{-12} mg. for the untreated animals and 293 and 298 x 10^{-12} mg., respectively, for dogs 146 and 148 before administration of the disulfide.

During the first month of anemia the total phospholipid was extremely elevated at times (853 and 642 x 10^{-12} mg.), producing average values of 476 and 432 x 10^{-12} mg., larger amounts than after three months in the anemic state when the values were 326 and 252 x 10^{-12} mg. per cell, respectively, for dogs 146 and 148. When the total phospholipid is separated into its individual components, salient differences are revealed. Cephalin was significantly elevated at times within the first month, whereas the choline phospholipids (lecithin plus sphingomyelin) reached both minimum and maximum values. There may be a close relationship between the presence of immature red blood cells, the elevated total phospholipid, and the individual phospholipid partition at the initiation of the anemia. Rapid erythrocyte destruction by the drug is followed by an outpouring of reticulocytes[8] which in this study occurred during the first month. With longer duration of the anemia the marked decrease in corpuscular cephalin and

elevated choline phospholipid indicate a definite change in the structure of the red blood cell. These results support recent views that hemolytic agents injure directly the structure of the mature erythrocyte, making it more susceptible to normal hemolytic processes in the body.[15]

TABLE V

LIPID DISTRIBUTION OF BRAINS

DOG	TOTAL LIPID	TOTAL PHOSPHOLIPID	FREE CHOLESTEROL	CHOLESTEROL ESTERS	NEUTRAL FAT	CEREBROSIDES	CEPHALIN	LECITHIN	SPHINGOMYELIN	WATER
Per Cent Wet Weight										
Normal:										
93	10.3	5.0	1.9	0.0	2.2	1.0	3.0	0.5	1.4	75.4
94	8.8	4.6	1.8	0.0	1.4	0.9	2.7	1.1	0.8	77.1
Anemic:										
146	9.1	4.6	1.8	0.4	0.6	1.6	3.0	0.7	0.8	77.1
148	11.0	5.0	2.3	0.1	1.2	2.2	2.8	0.9	1.2	74.8
Per Cent Dry Weight										
Normal:										
93	41.4	20.1	7.6	0.0	8.8	4.0	12.1	2.0	5.6	
94	38.2	20.0	7.8	0.0	6.1	3.9	11.7	4.8	3.5	
Anemic:										
146	39.8	20.1	7.9	1.8	2.6	7.0	13.1	3.1	3.5	
148	43.9	19.9	9.2	0.4	4.8	8.8	11.2	3.6	4.8	

In view of the neurologic manifestations which frequently accompany anemia, detailed lipid analyses and histopathologic studies* of the brains were made (Table V). No significant differences are evident in any of the lipid constituents except cerebrosides, which was slightly higher, and neutral fat, which was lower in the brains of the anemic dogs. Cerebroside values† for the anemic dogs were 1.6 and 2.2 per cent (wet weight) as compared to 1.0 and 0.9 per cent for normal dogs; neutral fat values were 0.6 and 1.2 per cent as compared to 2.2 and 1.4 per cent, respectively. The brains contained (on the wet weight basis) 5 per cent total phospholipid, 2 per cent free cholesterol, and negligible amounts of cholesterol esters. Cephalin constituted the major portion of phospholipid (60 per cent of the total), with the remaining 40 per cent about equally partitioned as lecithin and sphingomyelin.‡

Histopathologic examinations§ of the liver, spleen, and bone marrow of each animal showed that conspicuous changes had occurred with the anemia. The livers were found to be congested and contained pigment deposits. There was no evidence of an infectious process. The spleens contained a large amount of light brown pigment and the sinusoids were distended with red blood cells to such a degree that most of the normal splenic structure was indistinct. The appearance

*Each brain was divided into six separate sections, which were analyzed separately and then totaled for values on the entire brain. Specimens of the separate sections of each brain were taken for histopathologic examination by Gabriel Steiner, M.D., research professor of neurology and neuropathology, Wayne University College of Medicine, Detroit, and the results will be related with the chemical analyses in a later report.

†Cerebrosides were determined by a modification of the Kimmelstiel-Kirk[16] method.

‡Micro methods for lecithin, cephalin, and sphingomyelin developed in this laboratory are described in a separate publication.[18]

§The bone marrow smears were observed by Pearl Lee, M.D., hematologist, and the liver and spleen by Martha Madsen, M.D., pathologist, Children's Hospital of Michigan, Detroit.

was similar to the active stage of hemolytic icterus. Although x-ray examination* of the bones of the animals failed to reveal any noticeable changes, the bone marrow smears showed an increase in nucleated red blood cells and neutrophilic myelocytes, a picture of a hyperactive bone marrow. These results confirm those of Gruhzit[9] on the hemolytic nature of the disulfide anemia.

A review of the physical and chemical changes which occurred in the erythrocytes as the result of the disulfide anemia discloses further similarities to the abnormal red blood cells in active hemolytic icterus[2]; the enlargement of the cell accompanied by increases in fragility, water, and total base content is particularly analogous.

TABLE VI

LIPID AND PROTEIN COMPOSITION OF POSTHEMOLYTIC RESIDUE OF NORMAL AND ANEMIC DOG BLOOD

(Per cent of dry weight)

	NORMAL	AFTER 3 MO. OF ANEMIA
Dogs	93 and 94	146 and 148
Total lipid	16.9	11.6
Neutral fat	2.8	0.9
Free cholesterol	6.2	3.9
Cholesterol esters	0	1.7
Phospholipid	7.9	5.1
Cephalin	2.9	1.9
Lecithin	4.2	2.2
Sphingomyelin	0.8	1.0
Protein (N × 6.25)*	57.1	70.5
Amino aciid nitrogen:†		
Histidine	5.1	5.1
Arginine	11.5	9.9
Lysine	6.6	5.9
Tyrosine	2.1	2.1
Tryptophane	1.4	1.3

*Protein calculated on basis of total stroma.
†Per cent of total nitrogen of lipid-free stroma.

A more direct approach to the chemical changes which occur in the erythrocyte structure can be made by a study of the composition of the posthemolytic residue or stroma of the red blood cell.[3-5] In order to secure a sufficient amount of this material for detailed lipid and protein analysis, it was necessary to combine all the blood that could be drawn from the two dogs with N-propyl disulfide anemia at the end of the study. The lipid and protein analyses of the stroma from the combined blood of the anemic animals (146 and 148) are presented in Table VI, together with similar analyses on the stroma obtained from the combined blood of two untreated animals.

The stroma isolated from the cells in anemia had a total lipid content of 11.6 per cent as compared to 16.9 per cent for that of the normal dog stroma. Likewise, the neutral fat (which includes cerebrosides) and free cholesterol fractions were lower in the stroma from the anemic dogs. Noticeable, however, is the appearance of cholesterol esters. In the normal dog stroma no cholesterol esters could be detected. In the anemic stroma there was found to be 1.7 per cent cholesterol esters. Both cephalin and lecithin components of the phospholipid were lower, paralleling the lower phospholipid content, whereas the remaining

*See footnote † page 997.

phospholipid constituent, sphingomyelin, was approximately the same in the stroma from the normal and anemic dogs. The diminished cephalin content of the stroma is in accordance with that observed for the erythrocyte as a whole, but the decreased lecithin indicates that not all of this phospholipid within the anemic cell is combined with the stroma. The decreases in total phospholipid and free cholesterol, together with the appearance of cholesterol esters, are indicative of degenerative and retrogressive tissue changes[1] and imply a defective or impaired structure of the erythrocyte in the anemic state.

The total protein content, in contrast to the lipid, was lower in the normal dog stroma (57.1 per cent) as compared to the anemic stroma (70.5 per cent). Determination of the stroma content of histidine, arginine, lysine, tyrosine, and tryptophane, showed that on the basis of these amino acids, which account for 24 to 27 per cent of the total protein nitrogen, the normal dog stromal protein is similar to the anemic stromal protein and to that from the erythrocytes of other mammalian species[5]. It appears, therefore, that the disulfide anemia has no qualitative effect on the erythrocyte stroma protein. In view of the acidic nature of this type of protein[5] an increased proportion in the stroma, however, may be related to the elevated base content of the erythrocyte in the anemia.

SUMMARY

Correlated investigations of dogs with an experimental hemolytic anemia induced by N-propyl disulfide have included determination of hematologic, physical, and chemical characteristics of the blood; the chemical composition of the erythrocyte stroma; roentgenologic examinations of the bones and histopathologic observations of tissues.

As a result of the anemia, chloride, phospholipid and cholesterol esters were lowered in the serum, or plasma, and the neutral fat content was increased. The plasma lipid changes were similar to those observed in the hemolytic anemias of childhood and pernicious anemia and appear to be typical of all anemias.

In the N-propyl disulfide anemia the erythrocyte was larger and heavier, as shown by increased volume, weight, and diameter of the cells; an increase in water content accompanied by a lower specific gravity attended by greater fragility indicate that the anemia caused osmotic swelling of the red blood cells.

Elevated corpuscular base and lowered chloride contents, previously noted in anemias of man, point to an excess cation content or an increased amount of an undetermined anion in the erythrocyte of an anemic subject.

Marked alteration in the cellular structure of the erythrocyte in anemia is indicated by the changes in the lipid fractions; outstanding alteration was a striking decrease in cephalin content.

The chemical composition of the erythrocyte stroma confirms the altered red blood cell structure in anemia. The stroma, a complex of lipid and protein, was changed both quantitatively and qualitatively in its lipid content; the protein fraction of the stroma in anemia was similar in composition to normal stroma but larger amounts were present. It is significant, in view of the acidic nature of the stroma protein, that the red blood cells in anemia contained greater amounts of basic minerals.

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THE INFLUENCE OF THE PAROTID GLAND ON BLOOD SUGAR*

A Preliminary Report

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THE existence of a carbohydrate-regulating hormone, secreted by the parotid gland, has been postulated by several foreign observers. Hiki[1] and his collaborators obtained parotid extracts, which, when injected into dogs, caused marked and rapid hyperglycemia. Takacs,[2] on the other hand, reported a hypoglycemia in animals following the injection of ether and alcohol extracts of this gland. Rosenfeld[3] maintained that the hypoglycemia following the ligation of the parotid ducts is due to absorption of amylase or ptyalin, rather than to an internal secretion of an insulinlike substance. Thus biochemical studies have given rise to three differing conclusions concerning the action of parotid extracts.

Cahane and Cahane[4] in 1924 extirpated the parotid glands of two dogs. They reported subsequent hypertrophy and hyperplasia of the islands of Langerhans. Aunaps[5] reported cure of hyperglycemia of depancreatized dogs following parotidectomy. Ferretti,[6] on the other hand, stated that hypertrophy of the parotid glands is a compensatory mechanism; i.e., the parotids take on the function of the pancreas in the manufacture of insulin.

Injections of parotid extracts and surgical procedures on the parotid gland have had definite effects on blood sugars. These effects and the interpretations thereof have not been uniform.

The present study was undertaken, therefore, in order to study blood sugar levels and tissue changes in completely deparotidectomized animals.

EXPERIMENTAL PROCEDURES

Eight young male albino rats, weighing from 223 to 305 Gm., were used. A daily record of food consumption and weights was kept. Daily blood specimens were drawn at approximately the same time of day for a period of one week. The animals were not fasted prior to the drawing of blood, since there is no indication that greater precision in the estimation of blood sugars in rats could be secured by periods of inanition.[7] Four rats were chosen at random for the operative procedure and four rats were used as controls. Total bilateral parotidectomies were performed on rats Nos. 3 and 5; one-sixth of the right parotid gland of rat No. 7 was left in situ. In rat No. 1 both glands were removed except for one-eighth of the right gland around which several ties were tightly placed. The modified microcolorimetric method of Benedict[8] was used for the sugar determinations.

*From the Laboratories of the Delamar Institute of Public Health, Columbia University.
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RESULTS

Rat No. 3 showed a marked and significant decrease in the blood sugar from a preoperative mean of 110 mg. ± 13 mg. per 100 c.c. of blood to 22 mg. per 100 c.c. of blood (Table I). The blood sugar remained low for three days. On the fourth day the reading returned to the preoperative level.

TABLE I

BLOOD SUGAR READINGS*

CONTROL ANIMALS

ANIMAL NO.	PREOPERATIVE PERIOD		POSTOPERATIVE PERIOD	
	MEAN	STAND. DEV.	MEAN	STAND. DEV.
4	125	± 10	106	± 13
6	96	± 24	106	± 19
8	102	± 16	99	± 7
9	105	± 14	104	± 17

OPERATIVE ANIMALS

ANIMAL NO.	PREOPERATIVE PERIOD		POSTOPERATIVE PERIOD						
	MEAN	STAND. DEV.	1†	2	3	4	5	6	7
3	110	± 13	22	39	55	80	104	127	
5	103	± 17	36	35	46	42	66	83	107
1	109	± 15	364	148	82	77	67	63	90
7	97	± 15	132	105	111	89	133	127	131

*Sugar is expressed in milligrams per 100 c.c.
†Number of days after operation.

Rat No. 5 also showed a marked and significant decrease in the blood sugar, from a preoperative mean of 103 mg. ± 17 mg. per 100 c.c. of blood to 36 mg. per 100 c.c. of blood. On the seventh postoperative day the blood sugar was definitely in the preoperative level.

Rat No. 1 demonstrated a marked hyperglycemia. The blood sugar rose to 364 mg. per 100 c.c. of blood the first day postoperatively. Subsequent readings declined rapidly. By the seventh postoperative day the readings had returned to the preoperative level.

Rat No. 7 demonstrated no significant blood sugar changes throughout the course of the experiment.

Histologic Studies.—Sections were made of the liver, pancreas, adrenals, spleen, and testicles of all eight rats. No abnormal changes were found. The size, shape, and number of the islands of Langerhans of the operative animals were similar to those of the controls.

COMMENTS AND CONCLUSIONS

The postoperative blood sugars of rats Nos. 1 and 7 rule out the possibility that surgical trauma per se was responsible for the changes in the blood sugar levels. Decreased daily feedings or loss of weight alone could not have been the cause for these changes, since all four operative animals lost weight. Only the rats totally parotidectomized manifested significant blood sugar drops. We may conclude that these drops were directly due to the removal of a substance in the parotid gland which acts antagonistically to the action of insulin. Furthermore, the presence of even a minute fragment of parotid gland will prevent this fall in blood sugar, as sufficient antagonist can be elaborated by

such small fragments as were left in rat No. 7. Rat No. 1, on the other hand, demonstrated what may be considered an oversecretion of the parotid gland, due to irritations caused by ligatures passed through the gland. Subsequent tissue studies of the remnant of parotid gland in rat No. 1 revealed no structural changes.

We may conclude that the absence of parotid gland definitely lowers the blood sugar levels and, conversely, that the parotid secretes a substance that elevates the blood sugar.

Cahane and Cahane's[4] findings of hyperplasia and hypertrophy of the islands of Langerhans were not confirmed.

The significance of the reports of Aunaps[5] concerning the cure of hyperglycemia following parotidectomy of depancreatized dogs must remain in doubt unless a compensatory hypertrophy of unremoved pancreatic tissue in his animals can be ruled out.

The nature of the compensatory mechanism effecting the return of blood sugars to the preoperative levels is not yet understood. Further work along these lines is indicated.

SUMMARY

1. The blood sugars decreased markedly, though temporarily, in totally parotidectomized rats.

2. The blood sugar was not affected when approximately one-twelfth of the total weight of both glands was left in situ.

3. The blood sugar increased markedly, though temporarily, following ligation of part of the parotid gland.

4. All blood sugars returned to preoperative levels eight days following the operative procedure.

5. Histologic studies of the liver, pancreas, adrenals, spleen, and testicles of the deparotidectomized rats revealed no changes.

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SERUM CHOLESTEROL STUDIES FOLLOWING THE ADMINISTRATION OF ACETYL-β-METHYLCHOLINE CHLORIDE BY IONTOPHORESIS*

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THAT choline is important in cholesterol metabolism has been pointed out by Best and his coworkers[1-3] and by other workers. Best has shown that the administration of choline prevents the fatty infiltration of the liver in rats fed fat or cholesterol. Steiner,[4] working with 38 rabbits, some of which received cholesterol and others cholesterol and choline, found that the oral administration of 0.5 Gm. of choline daily to cholesterol-fed rabbits did not prevent the development of hypercholesterolemia when compared to the control group receiving cholesterol alone. He also concluded from this study that choline delayed, but did not prevent, experimental atherosclerosis. Baumann and Rusch[5] also observed that choline did not influence the cholesterol content of the blood in cholesterol-fed rabbits. Zinnitz[6] found that small amounts of cholesterol acetate inhibited the action of acetylcholine.

During the routine treatment of patients with acetyl-β-methylcholine chloride by iontophoresis, it appeared that a study of the serum cholesterol levels before and after treatment might be of interest. Accordingly, 12 persons were selected; 2 received 0.125 per cent acetyl-β-methylcholine chloride by iontophoresis and the remaining 10, 1 per cent of the drug by the same route. Serum cholesterol determinations were done before and at intervals of one-half, one, and two hours after the administration of the drug. Since the effect of the acetyl-β-methylcholine chloride occurs within a few minutes and persists for several hours, it was felt that the time intervals selected were of sufficient duration to demonstrate an effect, if any. Cholesterol determinations were done in duplicate by a modified Bloor's method.[7, 8]

RESULTS

Of the 2 persons receiving 0.125 per cent acetyl-β-methylcholine chloride, one showed an appreciable drop in serum cholesterol in one-half hour, with a marked rise in the one- and two-hour specimens, while the other showed a slight rise from the control value. Five of the ten persons receiving the 1 per cent solution showed an appreciable rise in serum cholesterol, varying from 11 to 27 mg. above the control levels at the end of the two-hour period. Of the remaining 5, 2 showed no change, while 3 showed a slight fall in blood cholesterol. The serum cholesterol in the 12 persons studied varied by an average standard deviation of ± 5.1 per cent, with a range of ± 1.9 to ± 9.7 (Table I).

*From the Department of Medicine, New York Post-Graduate Medical School and Hospital, Columbia University, New York.
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TABLE I

SUBJECT	SERUM CHOLESTEROL (MG. PER 100 C.C.)				MEAN	STANDARD DEVIATION (± %)	ACETYL-β-METHYL-CHOLINE (%)
	CONTROL	½ HR.	1 HR.	2 HR.			
D. B.	222	189	218	242	218	9.5	0.125
A. S.	200	214	211	208	208	2.6	0.125
A. C.	240	257	295	268	265	9.7	1
I. K.	218	195	216	236	216	7.2	1
R. McC.	170	169	175	190	176	4.2	1
L. C.	242	239	230	244	239	2.7	1
L. C.	229	259	248	240	244	5.5	1
A. H.	164	168	160	170	166	1.9	1
A. J.	160	140	150	152	151	3.5	1
M. B.	293	259	286	280	280	6.2	1
J. G.	272	268	286	291	279	4.5	1
C. G.	174	157	171	174	169	3.2	1
Average						±5.1	

DISCUSSION

That acetyl-β-methylcholine chloride exerts a physiologic action when given by iontophoresis has been amply demonstrated by Kovacs, Saylor, and Wright,[9] and by Kotkis and others.[10] These workers have demonstrated that the cations of acetyl-β-methylcholine chloride were responsible for the physiologic reaction due to this compound and that they could be driven into the body by iontophoresis only from the positive electrode of a galvanic source.

Although rises and falls occurred in serum cholesterol following acetyl-β-methylcholine chloride iontophoresis, on analysis these variations were found to lack statistical significance. Bruger and Somach,[11] reporting on the diurnal variations in blood cholesterol in a group of 18 normal persons, found the average standard deviation to be ± 8.0 per cent for a twenty-four-hour period, with an average ± 3.5 per cent standard deviation in the morning hours. Since the present experiments were carried out during the morning hours, the average standard deviation of ± 5.1 per cent found in this series was only slightly higher than the ± 3.5 per cent reported by Bruger and Somach.[11]

CONCLUSIONS

The administration of acetyl-β-methylcholine chloride by iontophoresis is accompanied by variations of the blood cholesterol, which, however, are not significant from a statistical standpoint.

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THE DISTRIBUTION OF DOSES OF RADIOACTIVE PHOSPHORUS IN RODENTS*

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WITH growing interest in the therapeutic uses of temporarily radioactive substances, it is important to learn the distribution of these in tissue and their rate of excretion. Radioactive phosphorus prepared† at the Harvard cyclotron by bombardment of red phosphorus was injected intravenously or intraperitoneally in mice, rats, and rabbits as aqueous solution of disodium acid phosphate. The dosages of radioactive material injected ranged from 5 microcuries to 17.5 microcuries in the mouse, from 30 microcuries to 239 microcuries in the rat, and from 50 microcuries to 84 microcuries in the rabbit.

Measurements were made by a Lauritsen type electroscope. The accuracy of measurements hinges on (a) the rate of deflection of the fiber on any given portion of the scale, and (b) the human error in determining the exact positions of the fiber in relation to the scale markings. This last error increases as the rate of deflection decreases. Hence values obtained for relatively active samples are more accurate than are those for samples of very low activity. The thickness of the aluminum foil window is of importance in the determinations. Ours is 0.0055 mm. thick, permitting passage of about 75 per cent of the soft β radiation of uranium oxide. The sensitivity of this electroscope is two divisions per minute per millicurie of radiation at one meter distance. We do not regard as accurate our measurements below 0.01 microcurie, although phosphorus of this degree of radioactivity may be detected in amounts of 3.5 by 10^{-14} Gm. About 10 per cent error must be allowed in all results by this method of measurement.

Although expressed in microcurie equivalents, the radiation is not actually comparable in biologic effect to the same amount of gamma radiation of radon. The tissue changes are now being studied.

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Radioactive phosphorus decays with a half life of 14.5 days. The radiation is made up of β particles,[1] having a half layer value of 0.5 mm. of aluminum.[2]

TABLE I

PER CENT PARTITION OF RADIOACTIVE PHOSPHORUS IN RATS

HOURS	2	48	120	168
Heart	0.3	0.4	0.3	0.3
Lung	0.1	1.2	0.3	0.6
Spleen	0.1	0.6	0.7	0.4
Liver	0.2	8.7	5.0	2.8
Kidneys	0.3	0.5	0.4	3.1
Bone	0	11.1	11.3	4.0

TABLE II

PER CENT PARTITION OF RADIOACTIVE PHOSPHORUS IN RABBITS

HOURS	24	48	120	168	240	2,592
Heart	0.6	0.6	0.7	0.4	0	0
Lungs	1.0	1.0	1.3	0.5	0	0
Spleen	1.4	0.4	0.2	0.2	0	0
Liver	11.1	11.4	9.1	5.3	0	0
Kidneys	0.7	0.6	0.7	0.6	0	Trace
Bone (2 femurs)	1.4	0	0	0	0	Trace
Blood (total)	2.8	2.6	0.14	0.13	Trace	0

TABLE III

PER CENT PARTITION OF RADIOACTIVE PHOSPHORUS IN MICE

HOURS	24	48	120	240	264	312
Heart	0.35	0.35	0.3	0.24	0.12	0.15
Lungs	0.7	0.7	0.4	0.2	0.24	0.2
Spleen	1.0	1.5	0.6	0.3	0.2	0.1
Liver	4.9	4.1	2.1	1.0	1.1	0.6
Kidneys	1.0	0.9	0.5	0.4	0.3	0.3

TABLE IV

URINARY EXCRETION OF RADIOACTIVE PHOSPHORUS IN THE RAT

HOURS	PER CENT
3	6.1
6	6.3
21	5.6
30	3.5
45.5	2.9

In Tables I, II, III, and IV the amounts of radioactive phosphorus present in the tissues are calculated in per cent of the dose originally injected, corrected for rate of decay. In the case of larger tissues measurements were made of weighed aliquot portions; in the case of smaller organs the entire organ was weighed and used. Blood and urine were dried before measurement;* other tissues were compressed to a thin film and dried, or ground and then dried, or ashed.

As may be seen from Tables I to III, the per cent partition varies in the different animals. These values are only indicative of general trends, and vary

*The addition of varying amounts of urine up to fivefold, causing variation in thickness of the dry films, did not affect the accuracy of measurement.

somewhat with dosages given as well as other factors. Some absorption occurs within two hours after injection (Table I). The bone naturally picks up a large amount and retains it long, traces being present in the rabbit after 108 days. Owing probably to their excretory activity, the kidneys retained a trace at the same time, although none could be found in the liver (Table II).

The partition varies in the different species, but in general the spleen, liver, kidneys, and bone show a material degree of absorption and retention. In view of the distribution of leucemic lesions, this partition is serviceable in concentrating radioactivity to some degree in the tissues often affected by leucemic infiltration.

Urinary excretion is rapid. Hevesy[3] found when tracer quantities of radioactive sodium phosphate are given by mouth, traces appear in the urine in twenty minutes. This urinary excretion is more marked early and falls off rapidly. About one-quarter the dose is excreted in the first forty-eight hours. Minute amounts were found in the urine of a rat ninety-eight days after subcutaneous injection.[3]

Table IV gives the rate of urinary excretion of radioactive phosphorus administered intraperitoneally or intravenously. In the first three hours, 6 per cent is excreted, and this rate continues with only slight diminution for twenty-one hours, falling to about one-half in the next day.

The fecal excretion could not be accurately measured in our animals, as slight contamination with urine could not be prevented. Tuttle, Scott, and Lawrence[4] found from 13 per cent to 26 per cent of orally administered radioactive phosphorus in the feces of leucemic patients. An unknown amount of this represents failure of absorption from the gastrointestinal tract.

There is variation in the per cent partition in various tissues, to a minor degree among individual animals of the same species and to a greater degree between different species.

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THE ESTER-HYDROLYZING ACTIVITY OF THE CENTRAL NERVOUS SYSTEM*

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INTRODUCTION

LIPIDS such as cephalin, sphingomyelin, lecithin, cholesterol, and related compounds form a major portion of nervous tissue. In the human brain and spinal cord, for example, fatty materials constitute about 50 per cent of the dry weight of the tissue,[1, 2] as compared with 15 per cent in muscle tissue.[3] It follows that lipid metabolism is of profound interest in the study of the biochemical processes that take place in the brain and nerves. Marked changes are known to occur in the compounds composing the myelin sheaths, especially during nerve formation and degeneration.[1, 4]

Naturally, lipolytic and ester-splitting enzymes may be expected to play an important part in the formation and transformation of nerve tissue lipids. Bearing out this deduction is the increase in lipolytic enzyme activity of the cerebrospinal fluid found in conditions in which severe brain tissue destruction is known to occur.[5]

Despite the fact that lipolytic enzymes may thus be expected to be present in nervous tissue in considerable amounts, earlier workers did not find appreciable lipolytic activity.[6-10] We surmised that this was due to the use of tissue extracts or autolysates. In the first instance they missed the desmoenzymes firmly attached to cellular structures; in the second, part of the enzymes were inactivated during autolysis.[11] We, therefore, used minced brain tissue carefully guarded against autolysis. By this technique we have been able to demonstrate marked lipolytic activity.

In this report we are presenting the results of quantitative estimations of the ester-splitting activity of various portions of the brain and spinal cord. This "enzyme map" of nervous tissue is of itself interesting in that the lipolytic activity has been found to vary with the anatomic structures and possibly, in turn, with the functions of the tissue. However, the main function of these normal distribution studies is to provide a standard of comparison for studies of diseased nervous tissue which are in progress.

METHODS

Preparation of the Tissue.—Throughout the experiments the brain and spinal cord of the monkey, *Macaca mulatta,* were used.† The monkeys had died of either

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pneumonia or tuberculosis so that they were not, strictly speaking, normal animals. However, the brains and cords appeared normal on gross dissection. In doubtful cases microscopic sections were made, but in no cases were abnormalities found. As soon as possible after death the brain and cord were removed and washed in a stream of distilled water to remove blood adhering to the surface. After stripping the leptomeninges, the cerebrum and cerebellum were separated from the pons and medulla oblongata. The following tissues were removed as carefully and completely as possible: cerebral cortex, centrum semiovale, corpus callosum, thalamus, and gray tissue of the caudate and lenticular nuclei. A slice of cerebral tissue (mixed cerebrum) containing cortex and subcortical white matter was also removed. No attempt was made to separate gray and white matter of the cerebellum, pons, or medulla, since the small size of the monkey brain made this impractical. In this study the pons and medulla, which were always combined, are referred to as the brain stem.

The tissues were minced separately in Petri dishes with a scalpel and spatula, formed into oblong cakes, and kept in the refrigerator at 0° to 5° C. Precautions were taken to minimize the length of time during which any tissue was exposed to room temperature. Tissue prepared in this way could be kept for a week or more with no significant loss of lipolytic activity.

Determination of the Lipolytic Activity.—The lipolytic activity was determined as follows: Substrate emulsions of tributyrin, ethyl butyrate, or olive oil, were prepared by shaking one part of substrate with 4 parts of 5 per cent gum acacia solution. The hydrolysis mixtures, made up in 50 c.c. Erlenmeyer flasks, preferably glass stoppered, contained 2 c.c. of substrate emulsion, 1 c.c. or more of buffer solution, and enough distilled water to bring the volume to 10 c.c. The amount and concentration of the buffer solution depend on the purpose of the experiment. A piece of tissue cut from the previously described cake and estimated to weigh between 250 and 300 mg. was used as the enzymic material. The exact moist weight of tissue was formed by weighing the hydrolysis flask before and after addition of the tissue. The flask was shaken vigorously by hand until the tissue was distributed in small pieces through the emulsion, and then incubated for twenty-four hours at 37° C. At the end of this period the reaction was stopped by the addition of 10 c.c. of 95 per cent alcohol, and the amount of acid formed was determined by titration with 0.05 normal sodium hydroxide, using 10 drops of 1 per cent phenolphthalein as indicator. Two kinds of control determinations were used. A substrate blank was determined by titrating a hydrolysis mixture after incubation without brain tissue. A tissue blank was determined by incubating tissue with buffer and water without substrating and titrating as previously the lipolytic activity was calculated as follows:

(1) Lipolytic activity per gram of tissue

$$= \left[\frac{\begin{matrix}(\text{c.c. 0.05 N NaOH}) \\ \text{Tissue and buffered} \\ \text{substrate}\end{matrix} - \begin{matrix}(\text{c.c. 0.05 N NaOH} \\ \text{Buffered} \\ \text{substrate}\end{matrix}}{(\text{weight of tissue used, in milligrams})} \right] \times 1000$$

—Autolysis value per gram of tissue.

The "autolysis value" in the foregoing formula was obtained as follows:

(2) Autolysis value per gram of tissue

$$= \left[\frac{\begin{matrix}(\text{c.c. of 0.5 N NaOH}) & -(\text{c.c. 0.05 N NaOH}) \\ \text{Tissue and buffer} & \text{Buffer}\end{matrix}}{(\text{weight of tissue used, in milligrams})} \right] \times 1000$$

Throughout the course of the experiments the phosphate buffer of Sörensen, as modified by Hastings and Sendroy,[12] was used. In order to avoid the arbitrariness involved in the use of a single buffer concentration, we studied the lipolytic activity at various concentrations of phosphate buffer. A stock solution of buffer of pH 7.9 was prepared at a salt concentration of 0.2 molar. By using 1, 2.5, 4, 5, or 8 c.c. of this stock solution in hydrolysis mixtures whose total volume was 10 c.c., it was possible to obtain phosphate buffer concentrations of 0.02, 0.05, 0.08, 0.10, and 0.16 molar, respectively. To obtain a buffer concentration of 0.0067 molar, 1 c.c. of fifteenth-molar stock solution was used. The activity was determined at pH 7.9 because this pH value is in the neighborhood of maximum hydrolysis of tributyrin and ethyl butyrate. Experiments were also made at the more physiologic pH value of 7.4 The difference in pH exerted no significant influence on the results obtained.

In all, 13 monkeys were used in the experiments reported. In view of the fact that the results obtained with individual animals showed relatively small variations, this number was considered sufficient.

RESULTS

All portions of nervous tissue tested showed considerable tributyrin and ethyl butyrate-splitting activity. This activity per gram of material was of the order of ten times that of blood serum. Neutral fat (olive oil) was not appreciably hydrolyzed.

The concentration of phosphate buffer in the hydrolysis mixture affected the rate and extent of hydrolysis. Within certain limits increased phosphate buffer concentration was accompanied by increased hydrolysis. In the case of ethyl butyrate this increase reached a maximum at 0.02 to 0.05 molar phosphate buffer concentration. Tributyrin-splitting activity reached a maximum at 0.16 molar.

Although the absolute ester-splitting power, as represented by the titration values in Table I and Figs. 1 and 2, varied from animal to animal, the relative order in which tissues from each individual animal split each ester was always the same. In other words, the ester-splitting power of the different tissues was characteristic of the tissues; the relative hydrolyzing power of various tissues under similar conditions of hydrogen-ion concentration, temperature, and phosphate buffer concentration was always the same. Thus at 0.16 molar phosphate buffer concentration the various tissues studied hydrolyzed tributyrin in the following order: cerebral cortex, mixed cerebrum, striatal gray matter, thalamus, cerebellum, stem, spinal cord, and white matter (Fig. 1). At lower phosphate buffer concentrations the difference in the tributyrin-splitting activity of the several tissues was not quite so distinct, yet gray and mixed tissues always showed more tributyrin-splitting activity than spinal cord and white matter.

TABLE I

HYDROLYSIS OF TRIBUTYRIN AND ETHYL BUTYRATE BY VARIOUS TISSUES OF THE CENTRAL NERVOUS SYSTEM

(Results are expressed as cubic centimeters of 0.05 normal sodium hydroxide used to neutralize the fatty acids formed.)

		CEREBRAL CORTEX	STRIATAL GRAY TISSUE	MIXED CEREBRUM	THALAMUS	CEREBELLUM	STEM	SPINAL CORD	WHITE MATTER
				0.16 Molar Phosphate Buffer					
Tributyrin	Max.	100.0	101.0	94.1		79.4	68.6	61.2	54.9
	Min.	82.7	72.9	82.6		76.8	51.5	48.5	46.8
	Avg.	92.3	85.3	89.4	80.0	73.7	60.8	53.8	50.7
Ethyl butyrate	Max.	25.7	26.0	24.9	23.8	19.0	18.7	11.2	13.0
	Min.	20.9	14.0	14.4	13.6	8.5	12.3	6.1	6.8
	Avg.	23.1	19.0	20.2	18.7	13.5	15.1	7.8	10.8
				0.10 Molar Phosphate Buffer					
Tributyrin	Max.	80.2	70.0	73.4	64.1	71.3	58.1	54.0	51.8
	Min.	68.7	52.5	63.1	57.0	58.7	43.1	45.0	33.5
	Avg.	75.4	63.6	68.4	60.5	64.9	51.5	48.8	44.3
Ethyl butyrate	Max.	28.4	25.2	23.4	20.7	19.2	20.4	11.7	13.9
	Min.	21.0	15.7	17.0	16.7	10.2	9.6	6.6	7.4
	Avg.	23.6	20.6	20.0	18.7	14.4	15.0	8.5	11.0
				0.0067 Molar Phosphate Buffer					
Tributyrin	Max.	14.9	15.7	17.1	17.3	16.0	16.3	13.2	14.6
	Min.	13.2	11.6	13.2	12.6	12.2	13.9	10.8	10.9
	Avg.	14.4	14.0	14.7	14.1	14.4	15.2	12.3	11.9
Ethyl butyrate	Max.	9.1	11.3	9.3	10.2	9.8	7.1	6.0	7.6
	Min.	6.6	6.9	5.6	7.0	4.5	5.7	3.0	3.4
	Avg.	8.2	8.6	7.3	7.9	6.5	6.5	4.4	5.3

Ester-splitting power of nervous tissue is strongest in gray matter. Phosphate buffer concentration has a notable effect.

On the other hand, ethyl butyrate-splitting activity at all phosphate concentrations remained in the order: cerebral cortex, mixed cerebrum, striatal gray matter, thalamus, stem, cerebellum, white matter, and spinal cord (Fig. 2).

It will be noted that the order of ethyl butyrate-splitting activity is somewhat different from that for tributyrin-splitting activity. The stem is more active than the cerebellum with respect to ethyl butyrate, and white matter is more active than spinal cord tissue. These differences were found consistently in all the animals studied.

DISCUSSION

It has been found in the present work that all the various tissues of the brain and spinal cord which we have studied have marked ester-splitting ability. The quantitative measure of this ester-splitting activity varies throughout the central nervous system and is a chemical characteristic of each tissue portion. In general, the ester-splitting activity is greatest in gray matter, least in white matter, and intermediate in mixed tissues.

The presence of lipolytic enzymes in the brain and spinal cord raises the question of the source and functions of these enzymes. Since white matter consists of nerve fibers and neuroglial cells, the enzymes of white matter are probably derived from the latter. Whether or not astrocytes, oligodendroglia, and microglia, contribute equally to the enzymatic activity of the white matter, is a question which cannot at present be settled. It is thought, on anatomic grounds,[13] that the oligodendroglia are related to the formation and maintenance

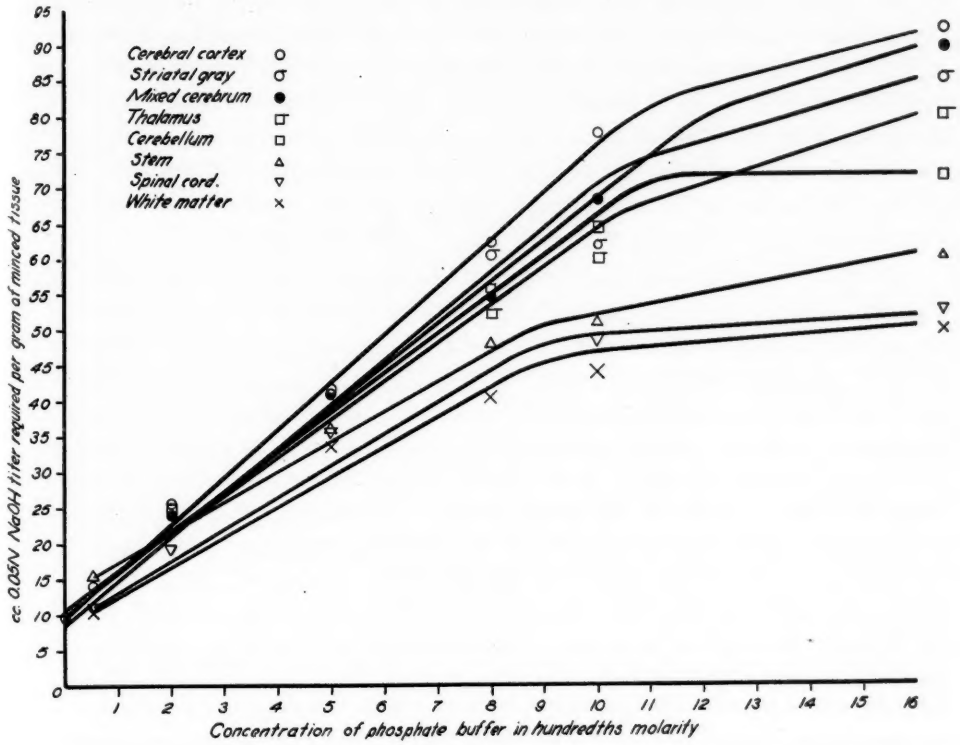

Fig. 1.—Curves showing the hydrolysis of tributyrin by various central nervous system tissues at different concentrations of phosphate buffer.

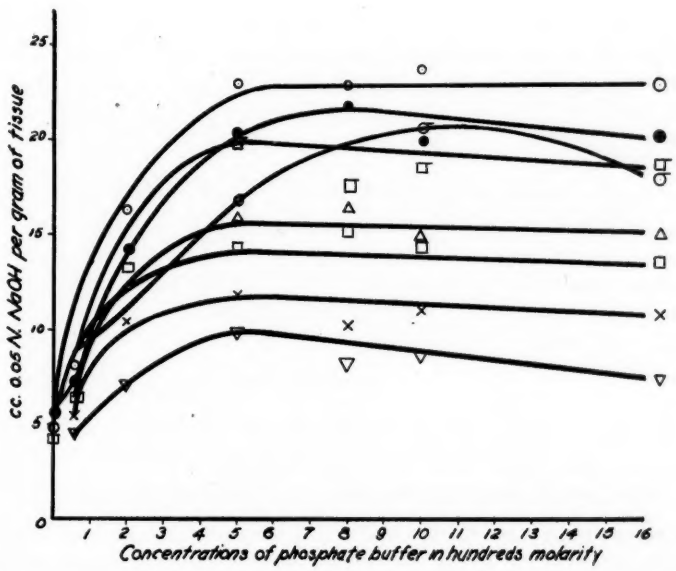

Fig. 2.—Curves showing the hydrolysis of ethyl butyrate by various central nervous system tissues at different concentrations of phosphate buffer. See Fig. 1 for key.

of the myelin sheaths of the nerve fibers. Since myelin regulation is an important part of the brain's lipid metabolism, it probably depends on the activity of lipolytic enzymes. It is possible, therefore, that the oligodendroglia take a more active part in lipid metabolism than do astrocytes and microglia. Gray tissue contains, in addition to neuroglia and fibers, large numbers of nerve cells. These cells are probably responsible for the greater lipolytic activity of gray tissue. So far no explanation can be offered for the relatively low activity of spinal cord tissue which contains all the structural elements.

It is both interesting and important to attempt to correlate the lipolytic activity of various parts of the brain with their lipid content. This can be attempted in view of the results obtained in the present study, and the previous work of Randall[2] on the chemical topography of the brain. Randall found that the various lipid constituents—total lipid, acetone-soluble lipid, total cholesterol, free cholesterol, phospholipid, phospholipid fatty acid, lipoid phosphorus, and lipid nitrogen—were higher in the white areas than in the gray areas, and intermediate in the mixed areas. The gray areas studied by Randall were frontal and parietal cortex and caudate nucleus; the white areas studied were corona radiata, frontal, and parietal white; the mixed areas studied were stem and thalamus. Randall also found that the mean water content was higher in the gray areas than in white, while the stem and thalamus again had intermediate values. It is apparent, therefore, that the relative degree of lipolytic activity can be inversely correlated with the lipid content of the various areas of the brain. Areas with greater lipid content appear to have relatively less lipolytic activity per unit of tissue than areas less rich in lipids. This relationship holds also for mixed areas, since the thalamus, which has greater lipolytic activity than the stem, has a smaller lipid content. It is also interesting to note that the order of lipolytic activity corresponds with that of the water content as far as regards white matter, stem, thalamus, and cortex. Unfortunately, Randall made no studies of the cerebellum and spinal cord, so that no light can be thrown on the apparently discordant results obtained for the ethyl butyrate hydrolyzing power of mixed tissues.

So far as pathologic conditions of the central nervous system are concerned, it would be interesting to study the lipolytic activity of various portions of the brain in degenerative diseases, both clinical and experimental, as well as in conditions brought about by avitaminosis or metrazol and insulin treatment. It might be possible, then, to correlate histopathologic changes with chemical changes.

SUMMARY

1. Fresh minced tissue of the brain and spinal cord of *Macaca mulatta* was found to have appreciable ester-splitting activity. Under standard conditions this activity was approximately the same in different animals of the species.

2. The ester-splitting activity varied throughout the central nervous system. However, tissues obtained from different portions of the central nervous system showed characteristic hydrolyzing activity.

3. The tributyrin-splitting power was greatest in the cerebral cortex, least in white matter, and intermediate in mixed areas. Mixed tissues rich in gray

matter, such as thalamus and cerebellum, were more active than mixed tissues rich in white matter, such as stem and spinal cord.

4. The ethyl butyrate-splitting activity was greatest in the cerebral cortex and least in the spinal cord. The cerebellum has less activity than other mixed tissues including the stem. White matter was less active than gray or mixed tissues with the exception of the spinal cord.

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LABORATORY METHODS

GENERAL

A NOTE ON THE BACTERIOLOGIC CULTURE OF BONE MARROW IN TYPHOID FEVER*

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THE localization of bacilli in the bone marrow during an attack of typhoid fever has been known for many years. Longcope[1] in 1905 noted the presence of large numbers of characteristic macrophages here, as well as in the Peyer's patches of the intestines, and also observed areas of necrosis in the marrow similar to those of the spleen and lymph nodes. Ludke[2] reported the persistence of typhoid bacilli in the lymph nodes and bone marrow of guinea pigs following intravenous inoculation and their proliferation in these situations after disappearance from the blood. Gay[3] further noted that the organisms were present in the marrow of infected rabbits not only after disappearance from the blood, but also from the lymph nodes and spleen. The clinical significance of this localization has been stressed in regard to the resultant leucopenia and neutropenia occurring during the acute phase of the disease and the osseous lesions which occasionally occur as sequelae of the infection. However, the value of marrow culture during the disease has been pointed out only within the past several years. In this communication we wish to record our experience with the bacteriologic examination of the marrow in three cases of typhoid and to comment briefly on the usefulness of this procedure.

Culture of the bone marrow in vivo has been closely associated with the increasing interest and exploitation of bone marrow biopsy in the blood dyscrasias. Cytologic examinations of the marrow have increased in number as a result of the introduction of simpler and more practical methods of securing samples of this tissue. Among the earliest attempts at marrow biopsy were those of Ghedini,[4] who first advocated tibial trephination for the diagnosis of leishmaniasis. This technique never became widely popular, although it was used by Zadek[5] and Peabody[6] in 1922 and 1927, respectively, in their classical studies on the marrow changes in pernicious anemia. Sternal trephination, introduced by Seyfarth[7] in 1923, reawakened interest in the diagnostic value of marrow biopsy. But it remained for the introduction of needle puncture of the sternum by Arinkin[8] in 1929 to popularize marrow study.

Within the past several years reports of attempts at bacteriologic and parasitologic examination of marrow in a variety of infections have appeared

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largely in European literature. Among the diseases so studied have been typhoid fever, brucellosis, subacute bacterial endocarditis, miliary tuberculosis, malaria, leishmaniasis, trypanosomiasis, and various septic states due to members of the pyogenic group of bacteria. Marrow examination in leishmaniasis seems to be accepted as a diagnostic procedure of recognized value.[9] In malaria Osgood[10] has reported the occurrence of larger numbers of parasites in the marrow than in the peripheral blood. Bock[11] concluded from a comparative study of arterial and venous blood cultures with marrow culture in a variety of septic states that the latter, while inferior to arterial culture, was considerably easier to obtain and was apparently superior to the more commonly used venous blood culture. Favorable experiences with this method in the diagnosis of brucellosis have been reported by Signorelli[12] and Baserga and Barbagallo.[13]

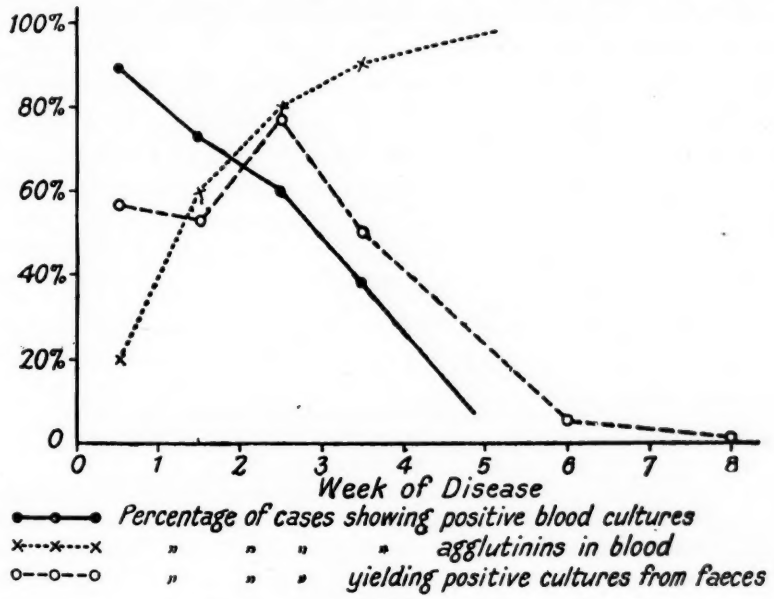

Fig. 1.
(Courtesy of Topley and Wilson.)

The earliest report of bacteriologic culture of bone marrow biopsy material in typhoid fever is contained in the little-known paper of Gerbasi,[14] who in 1925 obtained positive results from tibial bone marrow in thirteen cases. No further reports occurred until 1935, when Debré and co-workers[15] cultured the marrow obtained by sternal puncture in ten cases of typhoid during the first and second weeks of infection and obtained positive results in all instances. Further studies are recorded by Storti and Filippi,[16] Baserga and Barbagallo, and others. In all, approximately two hundred cases are included in these reports. Positive cultures were apparently obtained from patients not only in the first few days of the disease, but also as late as sixty to sixty-five days following the onset. It would appear from a scrutiny of this material that typhoid bacilli are present over a longer period of time in the marrow than in any other situation and that this tissue offers a constant reservoir from which positive cultures may be obtained.

The aim of diagnosis in typhoid fever has always been the actual isolation and identification of the organism from any possible source. The most fruitful sources for culture in active cases have been the blood and feces. Determination of the agglutinin content of the serum is an indirect method of diagnosis, the reliability of which depends on the demonstration of progressive increase in titer. Frequently titers of sufficient strength to be diagnostic do not develop before the end of the second week of the illness. Moreover, in this day of increasingly widespread use of prophylactic vaccination, the possibility of an "anamnestic reaction" of typhoid agglutinins to nonspecific hyperpyrexia must always be considered. Reference to Fig. 1 demonstrates that during the latter part of the second week of illness the diagnostic efficiency of current laboratory measures averages about 70 per cent. During this time, which has been called the "mute period" by Baserga and Barbagallo, marrow culture appears to have its greatest diagnostic usefulness.

The technique followed in the cases to be cited was as follows: The skin area over the body of the sternum at the level of the third interspace was cleaned with iodine and alcohol, and then anesthetized with 2 per cent novocain down to and including the periosteum. The needle used for puncturing the sternum was No. 18 gauge lumbar puncture needle with stylet in place, shortened to approximately 5 cm. according to the method suggested by Young and Osgood.[17] It was introduced at an angle of approximately 70°, and with a steady downward and somewhat rotatory pressure the anterior cortex was readily penetrated. After withdrawing the stylet, a tightly fitting 10 c.c. syringe was used to aspirate approximately 4 c.c. of sanguineous "marrow juice." This was transferred to media and treated in a manner similar to blood culture material. Most satisfactory growth was obtained on media containing selenite F.

Three patients with typhoid fever admitted to the University Hospital during August, September, and October, 1939, afforded an opportunity to observe the value of this procedure. Sternal marrow cultures were obtained once in each instance, i.e., on the fourteenth, nineteenth, and twenty-first days of the illness, respectively. Data concerning other bacteriologic and serologic studies in these cases, together with brief abstracts of the case histories, are cited.

CASE 1.—W. E. (Chart No. 27343), a white male, aged 14 years, was admitted to the Medical Service at the University Hospital on September 12, 1939, complaining of weakness, loss of appetite, and fever of two weeks' duration. Further questioning elicited complaints of more or less constant headache, marked constipation, and pain in the abdomen. The patient told of swimming in the harbor on several occasions some days prior to onset of illness. Examination revealed a very toxic, somewhat lethargic boy, with an admission temperature of 101.4° F. and a pulse rate of 88 per minute. Scattered small erythematous papules, which faded on pressure, were observed over the chest, back, and abdomen. A few sonorous and wheezing râles were heard over the bases of both lungs. The tip of the spleen was palpable 4 cm. below the left costal margin and the edge of the liver was likewise felt.

Aside from cellulitis of the right thigh which subsided uneventfully, the patient's course was entirely typical for typhoid fever, with persistence of hyperpyrexia for three weeks and gradual return to normal by lysis. Bone marrow culture on the twenty-first day of the illness was positive, and a simultaneous blood culture was negative. Agglutinin

titer, with both O and H antigens, never rose above 1:160. In this instance, the diagnosis having been established by prior positive cultures, bone marrow culture was merely of academic interest.

TABLE I

CASE 1. SEROLOGIC AND BACTERIOLOGIC EXAMINATIONS

DATE	DAY OF DISEASE	BLOOD CULTURE	URINE CULTURE	STOOL CULTURE	BONE MARROW CULTURE	AGGLUTINATIONS
9/13/39	16th	+				1:160 +
9/14/39	17th	–		+		
9/16/39	19th	–				
9/18/39	21st	–			+	
9/19/39	22nd		+			
9/21/39	24th	–				1:80 ++
9/29/39	31st			–		
10/ 1/39	33rd		–			
10/ 8/39	40th					1:80 ++
10/ 9/39	41st		–	–		
10/10/39	42nd		+			
10/11/39	43rd		+			

CASE 2.—A. F. (Chart No. 26969), a white female, aged 11 years, was admitted to the University Hospital on August 26, 1939, complaining of malaise and feverishness of thirteen days' duration. Illness was insidious in onset, with progressive drowsiness, fever, frequent headaches, and anorexia. Examination revealed a well-nourished, well-developed, somewhat toxic young girl, whose admission temperature was 105° F. and whose pulse rate was 110 per minute. Numerous "rose spots" were seen on the back, arms, and abdomen. A harsh mitral systolic murmur was heard. The spleen was not palpable.

TABLE II

CASE 2

DATE	DAY OF DISEASE	BLOOD CULTURE	BONE MARROW CULTURE	STOOL CULTURE	URINE CULTURE	AGGLUTININS (POSITIVE UP TO)
8/26/39	14th	–				
8/28/39	16th			–	–	1:320 +
8/29/39	17th			–	–	
8/30/39	18th	–		–		
8/31/39	19th		+	–	+	
9/ 2/39	21st	–		–		
9/ 5/39	24th					1:320 ++
9/ 6/39	25th	–		–	–	
9/ 8/39	27th			+		
9/11/39	30th	+		–		
		Relapse				
9/12/39	31st	+				1:320 +
9/13/39	32nd			–		
9/18/39	37th			–	–	1:160 +
9/21/39	40th	–				
9/26/39	45th	–		–		
9/28/39	47th				–	1:160 +
9/29/39	48th			–	–	
10/ 2/39	51st			–		
10/ 3/39	52nd	–				
10/ 9/39	58th	–		–		1:320 +++
10/16/39	65th	–		–		1:320 +
10/19/39	68th	–				
10/20/39	69th					1:320 ++

All bacteriologic examinations remained negative until August 31, when both bone marrow and urine cultures were found to contain *E. typhosa*. The agglutinin titer, however, two days after admission was positive up to a dilution of 1:320. Thus, in this instance, the

first definite bacteriologic evidence of typhoid fever was a positive marrow culture on the nineteenth day of illness. The patient's course in the hospital was marked by subsidence of fever within a week after admission, only to be followed a few days later by recurrence of hyperpyrexia, associated this time with several positive blood cultures. Recovery, however, was essentially uneventful, and the patient was discharged on October 22, 1939.

CASE 3.—D. R. (Chart No. 27929), a white female, aged 14 years, was admitted to the University Hospital on October 7, 1939, complaining of malaiase, fever, and listlessness which began on September 28. The illness was characterized by sudden onset with a chill followed by elevation of temperature to 104° F. On succeeding days there was recurrence of chills. On October 1, following therapy with quinine, there occurred a generalized wheallike eruption which lasted for twenty-four hours. Because of a known case of typhoid in the vicinity and continued hyperpyrexia, hospitalization was advised. Examination on admission showed a well-developed, well-nourished white female of 14 years, lying quietly in bed. The temperature was 100° F. and the pulse rate was 80 per minute. The remainder of the physical examination at this time was essentially negative. The laboratory studies were likewise noncontributory. Agglutination studies for typhoid were positive only up to titer of 1:80. Four days after admission, i.e., on the fourteenth day of illness, bone marrow culture was reported positive for *E. typhosa*. This was the first definite bacteriologic evidence of the etiology of the hyperpyrexia. It will be observed that blood cultures remained persistently negative in this case.

TABLE III

CASE 3

DATE	DAY OF DISEASE	BLOOD CULTURE	BONE MARROW CULTURE	STOOL CULTURE	URINE CULTURE	AGGLUTINATION
10/ 7/39	10th	–				
10/ 9/39	12th	–		–	–	1:80 +
10/11/39	14th		+			1:80 +
10/12/39	15th					1:160 + +
10/13/39	16th				–	
10/16/39	19th	–		+		1:80 +
10/19/39	22nd	–		–	–	
10/20/39	23rd					1:80 +
10/21/39	24th	–		+	–	
10/24/39	27th			–	–	
10/25/39	28th	–				
10/31/39	34th			–	–	
11/ 1/39	35th					1:80 +
11/ 3/39	37th			–	–	

COMMENT

The rationale for the bacteriologic examination of bone marrow exists in the fact that in this situation one may possibly secure capillary (arterial) blood as well as material rich in phagocytic reticulo-endothelial cells. These factors together are believed to offer greater probability of obtaining positive results than culture of venous blood taken at a distance from the locus of infection. In typhoid fever the growth and proliferation of organisms in the marrow has been known for many years. However, it remained for the development of a relatively simple technique, i.e., sternal puncture, to stimulate the clinical exploitation of this source of bacteria. The limited number of our cases does not permit at this time any broad generalizations as to the comparative value of this diagnostic procedure. However, our experience, as well as a consideration of the recent literature, seems to suggest that culture of the marrow offers a reliable and constant source of organisms during the acute disease from the earliest

days of the illness to the end of the febrile period. The procedure is, therefore, of value in early diagnosis, but appears to have its greatest usefulness at a time, roughly between the tenth and fifteenth days of the disease, when current diagnostic laboratory procedures are positive in only about 70 per cent of the instances. The further value of this technique, moreover, appears to us to lie in its possible extension to other types of infection associated at one time or another with bacteriemia.

SUMMARY

1. Bacteriologic culture of sternal marrow obtained by needle puncture on the fourteenth, nineteenth, and twenty-first days of the illness in three cases of typhoid fever was uniformly positive.

2. Further work seems indicated to establish the value of this procedure in typhoid fever and other infections.

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IMMUNOLOGIC TEST FOR HUMAN BLOOD AND HUMAN PROTEIN IN STOOLS*

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IT IS well known that protein is generally absent in human stools but that there are occasional cases in which presence of protein may be shown by chemical methods.

It is of the greatest importance to determine whether such protein found in the stool is derived from food not sufficiently digested or from the human body because of the vastly different significance of these findings. Hence it seems of practical value to have a readily available method by means of which protein in the stool can be easily identified as being derived from the human body.

Since we have no special chemical test for human blood protein, we must make use of immunochemistry. Injecting human serum into rabbits, the antiserum developed after a certain length of time will react in a specific way, giving a specific precipitate with human serum protein.

To prepare this antiserum (precipitin), human serum free of hemoglobin is diluted ten times with saline and after being slightly acidified (to pH 6.5) is absorbed on the same volume of aluminum cream following the procedure of Hektoen and Welker. After centrifuging until no protein is left in the supernatant fluid, the fluid is discarded and the remaining precipitate is mixed with saline so that 1 c.c. of this emulsion corresponds to 3 c.c. of diluted or 0.3 c.c. of original serum. Twenty cubic centimeters of this emulsion are injected at different places in the muscles of the hind legs of a rabbit forming a few deposits from which the protein may be absorbed into the blood. The antibody has usually reached a maximum at the end of two weeks. After a test has shown a titer of 1:100,000 with human serum, the rabbit is bled to death and the serum is ready for the test. It is kept in the refrigerator for further use.

For the stool precipitin test the stool is well mixed so as to secure a thorough distribution of the protein in the stool. As it is rather difficult to secure a completely uniform stool mixture, several portions of the well-mixed stool may have to be examined. The stool specimen is thoroughly mixed with five parts of 0.9 per cent sodium chloride solution. The stool suspension is centrifuged, the supernatant fluid is filtered through good filter paper, and the filtrate is returned to the filter until the filtrate appears clear. Clearing the filtrate by use of adsorbents, such as kaolin or Fuller's earth, or by using a Berkefeld filter is not admissible, as by such procedure protein would be removed. As soon as the filtrate is clear it is ready for the test.

For the test we employ very small test tubes, 3 mm. in diameter and 1 inch high. Pipettes with very fine points are made from glass tubing. With one of

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these pipettes we place in the tube a small amount of stool filtrate so that the tube is about one-third filled. With another fine pipette we lay about the same amount of antiserum below the stool filtrate, taking care that the point of the pipette goes through to the bottom of the tube. If human protein is present, a white ring—the specific precipitate—will develop at the border of the two layers. In case the protein content is rather high, the ring will appear immediately. If no ring appears within one hour, the test is negative.

The reaction is specific for human protein and is negative for protein from food or bacterial bodies. Hence if positive with stool extract, it is specific for colitis, exudates, or bleeding from the colon.

For differentiating between exudate and bleeding the absence or presence of human blood corpuscles must be determined. This can also be done immunologically, testing by the precipitation method for human hemoglobin.

To prepare an antiserum for human globin, rabbits are injected with a solution of human hemoglobin. This is prepared by hemolyzing thoroughly washed human corpuscles by adding slowly a small amount of ether (10 to 12 drops to 100 c.c.), shaking two to three minutes and repeating this procedure a few times until the corpuscles are completely hemolyzed. This solution is mixed with three parts of aluminum cream and centrifuged. The clear red solution devoid of serum protein and containing the hemoglobin may be used as an antigen for producing a precipitin specific for human globin. Injecting a rabbit every third day with increasing amounts of this solution, 1 c.c., 2 c.c., 3 c.c., 5 c.c., 8 c.c., and 10 c.c., one week after the last injection the serum may give the precipitin test with a hemoglobin solution 1:100,000. The test is made in the same way as described before for proving the presence of human protein. Positive test of the stool extract with this antiserum demonstrates the presence of human globin and thus of hemoglobin, and shows, together with the positive test for human blood protein, that bleeding occurred some place in the intestine.

CONCLUSIONS

1. By precipitin reaction, using as antibody a specific human serum-rabbit antiserum, the presence of human blood protein may be detected in human stools.

2. Using as antiserum a human hemoglobin-rabbit antiserum, the presence of human corpuscles or derivatives thereof can be demonstrated.

3. Positive precipitin reaction of stool extract with both serum antiserum and globin antiserum is characteristic for bleeding, whereas precipitin reaction with serum antiserum and negative test with globin antiserum will indicate an exudate from the bowels as produced by an exudative process.

4. Testing with dilutions of the feces extract will give a rough quantitative estimation.

ANTHRAX IN SHAVING BRUSHES*

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THE finding of anthrax spores in toothbrushes, hairbrushes, or shaving brushes occurs infrequently. Smyth (1939) reviews a twenty-year survey of anthrax in the United States.[1] In his tabulation of "Cases and Deaths by Source of Infection" appear 2,044 cases and 136 deaths where the source of infection is other than "transportation," "agricultural," or "professional." Such definite commercial commodity data have been adapted to Tables I and II.

Table I

Anthrax Cases and Deaths Having Definite Trade Commodity Sources of Infection 1929-1938 Inclusive

COMMERCIAL COMMODITY	CASES	DEATHS	TOTAL CASES (PER CENT)	TOTAL DEATHS (PER CENT)
Hides and skins	1,113	58	54+	43–
Wool	565	34	28–	25
Hairbrushes	207	18	10+	13+
Shaving brushes	159	26	8–	19+
Total	2,044	136 (7 –%)		

+ signifies slightly more than the whole number given.
– signifies slightly less than the whole number given.

Table II

Comparison of Commercial Commodity Sources of Infection With Deaths Reported

COMMERCIAL COMMODITY	CASES	DEATHS	DEATHS TO CASES
Hides and skins	1,113	58	5+
Wool	565	34	6+
Hairbrushes	207	18	9–
Shaving brushes	159	26	16+

Tables I and II emphasize that, although shaving brushes represent slightly less than 8 per cent of this grouping of industrial cases, yet the highest percentages of deaths occur in the shaving brush source group. The higher vascularity of tissue exposed and the minute nicking of the epidermis during shaving both favor the introduction of the organism into a suitable environment for propagation.†

The isolation and identification of *Bacillus anthracis* are simple laboratory tasks. However, in the examination of suspected shaving and similar brush material, a "hunch" in our own experience seems worthy of reporting, as evidence of extending sampling a bit further than in the examination of the hairs or bristles of the brush proper.

Smyth contends, "The latest outbreak of shaving brush cases is due to shipments of cheap brushes made from 'sterilized' horsehair, imported from Japan." We were interested in the possible presence of such brushes in our community.

*From the Public Health Laboratories, Los Angeles County Health Department, Los Angeles.[2]

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†Concerning shaving brushes, Smyth states, "They have a fatality rate of from 50 per cent to 60 per cent."

Early in 1939 our sanitary inspectors were asked to find out if "Imperial, Sterilized Japan, 332" shaving brushes were in stock for sale in our local stores. Only one such brush was found. It was purchased in a 5 and 10c store, by inspector W. C. Weymouth of our department. The brush was sealed well in cellophane, and was an attractive appearing piece of merchandise. Much to our surprise, this single sample yielded anthrax organisms by direct culture and from guinea pig inoculations. This brush was the only one of this brand found still unsold.

Radio and press requests were made in order to contact purchasers of this brand of brush. Nine used brushes were submitted to us as a result, one being received from a mountain town about 350 miles distant from Los Angeles.

These brushes had been in active use by the owners; none yielded anthrax organisms from the brush portion. Only one of the owners felt that he had experienced an "infection." This man stated that he had bought the brush about a year before. He "now recalled" that he had probably experienced an anthrax infection following the initial use of the brush. Our interested inquiry as to what kind of symptoms he experienced brought the reply of "no sores, but a peculiar feeling under the skin." We asked "What sort of peculiar feeling?" The answer was "Oh! like ants crawling around under the skin, you know, ant tracks!" Thus is our terminology sometimes misinterpreted by the layman.

We did not expect to recover anthrax from these used brushes, but examination of the merchandise led to an attempted innovation in such an examination.

The handles of hard wood were broken open, and the resinous brittle gum was crushed, liberating the ends of the hairs from their protective seal. The handle end of the hair was then subjected to culture and cavy procedures. One of the nine yielded a luxuriant growth, and gave typical post-mortem evidence in a cavy; the heart-blood culture yielded pure colonies of anthrax.

Inasmuch as this brush had been purchased either late in 1937 or early in 1938 and had been in constant use up to March, 1939, it is interesting to conjecture on whether or not the actual brush tuft portion had been effectively sterilized after the handle and hair had been put together, or had the user simply been fortunate is not contracting anthrax during the period of first use, before the spores were removed as a result of the daily washing out of the brush?

SUMMARY

Breaking open the handles of bristle or hairbrushes, removal of the handle binder from the hairs, and subjection of this portion of the hair to cultural and animal procedures for the demonstration of anthrax, yielded a positive result in one of nine used shaving brushes of Japanese manufacture.

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LABORATORY STUDIES IN THE FALCIPARUM MALARIA OF DRUG ADDICTS*

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THE literature contains many clinical pathologic studies concerning the disease of malaria. The recorded observations deal with the various types of malaria resulting from natural mosquito infection, and more recently, from the malaria induced for the therapy of nervous system syphilis. Except for the notes on "Laboratory findings" contained in Helpern's paper,[1] we have been unable to find any clinical pathologic studies of the falciparum malaria of drug addicts resulting from the common use of hypodermic syringes for intravenous heroin administration.

Falciparum malaria in drug addicts is now a well-established and recognized entity in New York City and probably also occurs in considerable numbers in other large metropolitan areas. Studies on the epidemiology and clinical aspects of the disease are reported elsewhere.[2]

During the past six years we have had the opportunity of observing well over two hundred drug addicts suffering from falciparum malaria contracted as a result of the common use of hypodermic syringes for intravenous heroin administration. All clinical varieties of falciparum malaria were encountered, including fatal cerebral, gastrointestinal, and hemoglobinuric syndromes. In many cases detailed laboratory studies were carried out. It is the object of this paper to present the findings of these studies.

MATERIAL AND METHODS

The patients studied in this series were drug addicts in whom a proved diagnosis of falciparum malaria was made. They were hospitalized, observed, and treated on the wards of the Third Medical Division and the Medical Service of the Psychiatric Division of Bellevue Hospital, New York.

Blood counting was done with the aid of certified blood pipettes. Hemoglobin was determined by an acid hematin method using a Klett-Newcomer disk colorimeter. The mean corpuscular volume was determined by the Wintrobe method.

The nonprotein nitrogen was done by the micro-Kjeldahl method, and blood sugar by the Folin-Wu method; serum bilirubin by the Ernst-Förster method; and plasma proteins by the tyrosine method. The protein in the spinal fluid was precipitated and determined by the micro-Kjeldahl method.

Urobilinogen was determined by the dilution method of Wallace and Diamond using Ehrlich's reagent (paradimethylaminobenzaldehyde).

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TABLE I

HEMATOLOGIC DATA ON ADMISSION

Red Blood Cells	
R.B.C. millions per c.mm.	Cases
1-1.9	2
2-2.9	19
3-3.9	19
4-4.9	27
Over 5	7
Hemoglobin	
Grams per 100 c.c.	Cases
Less than 5	2
5-10	32
10.1-15	36
Over 15	3
Mean Corpuscular Volume	
Cubic microns	Cases
Less than 80	6
80-94	9
Above 94	9
White Blood Cells	
W.B.C. thousands per c.mm.	Cases
Less than 5	9
5-10	35
Over 10	31
Lowest count 3.25	
Highest count 46.00	
Monocytosis (above 15 per cent)	Cases
Present*	12
Absent	63

*Ingested pigment frequently found.

HEMATOLOGIC OBSERVATIONS

Red Blood Cells, Hemoglobin, and Mean Corpuscular Volume.—In the hematologic tables one sees that in about half the individuals studied, considerable anemia existed. In 28 per cent the red blood cell count ranged between 1 and 2.9 million cells per cubic millimeter and in 46 per cent the hemoglobin was reduced below 10 Gm. per 100 c.c. On the other hand, in many individuals, despite severe malarial infection, little or no anemia existed.

The mean corpuscular volume was determined in 24 patients. The results obtained in 15 showed that the size of the red blood cells is in the normal range. In 9 patients, however, there was definite macrocytosis. Since there is no corpuscular enlargement associated with the development of *P. falciparum*, another explanation must be sought for the macrocytosis. Certainly these cases are complicated by dietary insufficiency and possibly by liver damage. These factors may account for the phenomenon of macrocytic anemia. It is of interest to note that Fairley and Bromfield,[3] in studying the average diameter of the red blood cells in 14 patients with *P. falciparum* infection (not drug addicts) by the halometer, found them all within the normal range. He doubts the existence of true megalocytic anemia in uncomplicated cases. Certainly the disease in the drug addict is not simple, uncomplicated malaria. Prolonged dietary inadequacy associated with chronic drug addiction, in many cases alcoholism and intrinsic

TABLE II

BLOOD CHEMICAL DATA ON ADMISSION

Nonprotein nitrogen

Milligrams per cent	Cases
20-35	41
36-50	13
51-100	8

Blood sugar

Below 100	23
101-150	23
151-200	6

Serum bilirubin

0.5-1.0	17
1.1-2.0	15
Above 2.0	18

Van den Bergh (direct reaction)

Result	Cases
Immediate	18
Delayed	16
Negative	13

Plasma Proteins Studied in 19 Cases

Serum albumin*										
Gm. per 100 c.c.	3.0	3.7	3.4	2.6	3.4	3.5	2.7	4.1	2.4	
Serum globulin	3.5	3.4	3.1	3.2	2.8	2.8	2.0	3.4	3.5	
Total	6.5	7.1	6.5	5.8	6.2	6.3	4.7	7.5	5.9	
Serum albumin	2.4	2.1	3.0	4.0	2.3	2.4	2.8	2.2	5.3	2.5
Serum globulin	2.4	2.2	1.6	2.5	2.2	2.1	2.7	1.6	2.2	2.8
Total	4.8	4.3	4.6	6.5	4.5	4.5	5.5	3.8	7.5	5.3

Analysis of Plasma Proteins

Serum albumin

Grams per 100 c.c.	Cases
2.0-3.0	12
3.1-3.7	4
4 or over	3

Serum globulin

Grams per 100 c.c.	Cases
1.5-2.8	13
2.9-3.5	6

Albumin-Globulin ratio

Normal	3
Reduced or inverted	16

*Normal values Albumin 4.0-5.3.
Globulin 1.8-2.7.
Total 6.5-7.9.

or malarial liver disease, may constitute factors in the mechanism of the anemia seen in these addicts, in addition to that directly dependent on the malarial infection itself.

White Blood Cell Count and Monocytosis.—From a diagnostic point of view, the white blood cell count and the differential formula, especially with reference to the number of monocytes, is seen to be of little value. In our series the white blood cell count was found elevated in 31 cases, normal in 35 cases, and less than normal in 9 cases. Monocytosis was seen only in 12 of 75 cases in which a differential count was done. Unfortunately this finding, so much stressed in texts, would appear to be of little value as a diagnostic aid. The presence of ingested pigment, however, is strong presumptive evidence of malarial infection.

BLOOD CHEMICAL OBSERVATIONS

Nonprotein Nitrogen.—The blood urea is frequently elevated in falciparum malaria. The nonprotein nitrogen was determined in 62 patients. Values between 51 and 100 milligrams per cent were found in 8. In two of these patients kidney disease (acute or chronic glomerulonephritis) was known to exist, but in the remaining 6 the elevation was felt to be associated with the malarial process and was dependent on one or more of the following: dehydration, vomiting, anoxemia, anemia, and hemoconcentration. In the cases in which recovery followed treatment, but no kidney disease existed, the values returned to normal very quickly.

Blood Sugar.—The blood sugar was found elevated above 150 milligrams per cent in 6 patients. In these one or more of the following was present: severe vomiting, hemoconcentration, high fever, anemia, or impaired liver function. We feel that one or more of these factors in our series may explain temporary derangement of the carbohydrate regulatory mechanism.

Serum Bilirubin.—Values of serum bilirubin up to 1.0 milligram per cent were considered normal. Sixty-six per cent of the persons studied exhibited hyperbilirubinemia. In the cases in this series, whenever the height of the serum bilirubin was considerable, the van den Bergh reaction was of the direct immediate type. The higher levels were associated also with moderate anemia and clinical icterus, as well as with urobilinuria.

Plasma Proteins.—The total proteins were reduced below 6.0 Gm. per cent in 11 of 19 individuals whose plasma proteins were studied. In the main this reduction, as well as a lowering or inversion of the albumin-globulin ratio, was due to the depression in the value for serum albumin. The latter values were considerably below normal in at least 16 of the total number studied. Values for the serum globulin, on the other hand, were essentially normal, although definitely increased in 6 patients.

SPINAL FLUID

It is surprising that in the face of the severe involvement of the brain, which occurred so often, relatively few changes were observed in the composition of the spinal fluid.

SEROLOGIC DATA

The Wassermann reaction was strongly positive (4 plus) in 15 patients, weakly positive in 2 and doubtful in 3. Even though one may concede a greater incidence of syphilis among drug addicts, one would hardly expect such a high percentage of syphilis if the positive Wassermann is interpreted as evidence of syphilitic infection. In 8 persons in whom the Wassermann was 4 plus at the height of the malaria, the reaction was negative after recovery. Two weakly positive and two doubtful reactions likewise became negative after recovery from active malaria. That the Wassermann reaction in malaria may be positive in the absence of syphilis is well appreciated in the tropics and elsewhere[4] and is re-emphasized in this series.

Henry's Serum Flocculation Test for Malaria (Chorine Modification).[5]—This test was positive in the 25 patients in whom it was carried out. We performed it only as a matter of academic interest, since in all cases the diagnosis

TABLE III

SPINAL FLUID DATA

Clear	23
Grossly bloody (not technical) not xanthochromic	2
Xanthochromic	2
White blood cells (more than 10 per c.mm.)	2
Globulin present	10
Globulin absent	15
Reducing substances present	25
Reducing substances absent	0
Wassermann negative	25
Wassermann positive	0
Colloidal gold curve normal	25
Colloidal gold curve abnormal	0

Chemical—Quantitative

Protein—mg. %	Cases
5-15	6
16-30	1

Sugar	Cases
40-60	3
61-90	5

Chlorides	Cases
Below 700	5
700-750	4
Above 800	1

TABLE IV

SEROLOGIC DATA

Blood Wassermann Reaction

Result	Cases
Strongly positive	15
Weakly positive	2
Doubtful	3
Negative	46

Henry's Serum Flocculation Test for Malaria (Chorine Modification)
Performed in 25 cases

Dilution	Cases
1:200	8
1:450	10
1:1,000	5
1:1,200	2

Positive serum is one showing flocculation in dilution of 1:200 or higher.

TABLE V

Urinary Findings

Examination	Positive
Albumin	27
Acetone	5
Reducing substance	5
Bile	7
Casts	12
Red blood cells in excess	10
Methemoglobin	2

Urobilinogen

Dilution	Cases
1:20	17
1:40	13
1:80	13
1:160	12
1:320	6
1:1,000 and above	2

was established previously by an examination of a stained blood smear. The test may be of value if plasmodia cannot be found, as in latent or chronic or unusual forms of malaria.

URINARY FINDINGS

The urinary findings were not striking. Albumin, acetone, bile, reducing substance, and methemoglobin were present in small amounts, and red blood cells and casts in small numbers. Any or all of the findings enumerated in Table V may be explained by the malarial infection per se. On the other hand, cerebral involvement, vomiting, starvation, dehydration, diarrhea, ingestion of other drugs, hemorrhage, and intravascular hemolysis, which operated in many cases in this series, might equally serve to explain the urinary findings here reported. The output of urinary urobilinogen is also represented in Table V. It is of note that in 46 persons values of 1:40 dilution or higher were found. Hemoglobinuria appeared in the two patients with clinical blackwater fever.

SUMMARY

The clinical pathology of falciparum malaria of drug addicts is presented. None of the laboratory findings, other than direct blood smear examination, is diagnostic of the disease. On the whole, the laboratory observations are those seen in natural and induced malaria. The outstanding findings are:

1. Anemia, which may be macrocytic.
2. Hyperbilirubinemia with an altered van den Bergh reaction.
3. Hypoproteinemia, due on the whole to depression in the value of the serum albumin and resulting frequently in reduction or inversion of the albumin-globulin ratio. The serum globulin may be considerably elevated.
4. Relatively high incidence of positive Wassermann reactions.
5. Positive Henry's flocculation test.
6. Increased urinary output of urobilinogen.

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A COTTON-PLUG WRAPPING MACHINE FOR BACTERIOLOGIC CULTURE TUBES AND BOTTLES

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IN MANY bacteriologic laboratories large numbers of cotton plugs are required for use as stoppers in culture tubes, flasks, and bottles. The demand for such plugs is particularly large where water samples are tested by the Most Probable Number method. It has been found that considerable experience is necessary before an individual becomes sufficiently skilled to make good plugs by hand methods at even a reasonable rate. It seemed probable to us that a simple machine could be constructed that would produce better plugs at a faster rate. Such a machine was put together from miscellaneous materials at hand in our laboratory. It consists of the following parts:

1. A one-thirtieth horse power, induction wound, electric motor.
2. A mandrel fashioned from a sixteen-penny finishing nail which was filed to a triangularly tapered point, and then polished with fine sandpaper.
3. A gear-operated speed reducer connected directly with the motor that turned the mandrel approximately 170 r.p.m.
4. A foot switch of the instantaneous action, circuit-breaker type.

The motor and speed reducer are one unit which was originally designed to revolve a brush used in washing test tubes. The chuck, in which the test tube brush was formerly placed, is used to hold the mandrel. In order to adapt this machine for use as a test tube plug wrapper, it was mounted firmly on a work bench. This was accomplished with two "C" clamps purchased at a hardware store.

Fashioning of the mandrel was relatively simple. A sixteen-penny nail was placed in a vise and the head was filed off. The ends were then reversed in the vise and the nail was filed to a triangularly tapered point which was about three-fourths as long as the plug desired. It is extremely important to polish the mandrel because file marks or other irregularities will catch cotton fibers and make the plug difficult to remove. The polishing operation should be carried out with lengthwise strokes, using very fine sandpaper. The finished mandrel mounted in the chuck on the electric motor is shown in Fig. 1.

A sensitive, instantaneous action foot switch is necessary to provide positive control of the motor. Our first switch was made from a doorbell push button mounted between two boards. This has proved satisfactory. A similar switch made for photograph enlarging machines could be adapted for use with this machine. Such a switch can be purchased at most photographic supply stores.

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The operation technique depends somewhat on the type of plug desired and on the operator. We have found the following system to be simple and fast:

1. A roll of cotton is separated into layers of suitable thickness. For test tube plugs an average roll is separated into three thicknesses, each of which is cut into four strips (one strip will make several plugs).

2. One end of a cotton strip is placed over the mandrel's tapered end, as is shown in Fig. 2. The machine is started by depressing the foot switch, and the cotton is fed onto the plug until sufficient material has accumulated. The plug is shaped between the thumb, index finger, and middle finger of the left hand.

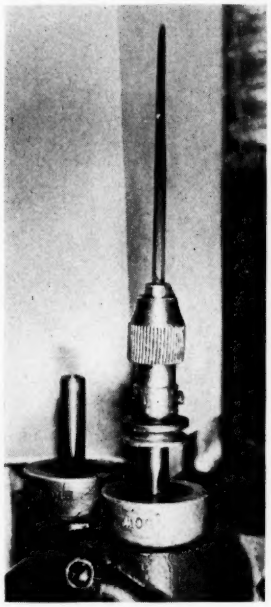

Fig. 1.

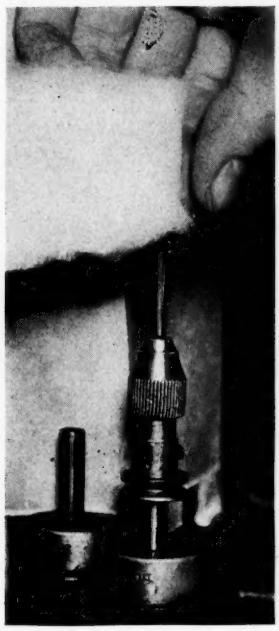

Fig. 2.

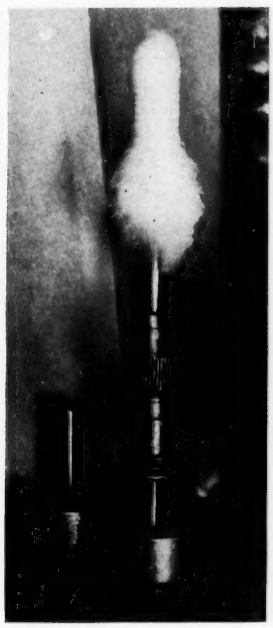

Fig. 3.

Fig. 1.—View of chuck and mandrel showing manner in which mandrel is formed from a sixteen penny finishing nail.

Fig. 2.—View from the operator's position showing cotton being fed onto the mandrel. A foot switch (not shown) starts and stops the electric motor.

Fig. 3.—The finished plug is easily pulled from the mandrel when the motor is stopped.

3. At this point, with the machine still running, the strip is torn from the plug which is then finished with the fingers of the left hand. Varying pressure of the fingers will vary the tightness and hardness of the finished plugs. A finished plug is shown in Fig. 3.

4. The formed plug is now guided into a test tube, the motor is stopped, and the plugged tube is removed from the mandrel. If an extremely hard plug has been made, it may be necessary to twist the plug about one-quarter turn opposite to the direction of formation rotation in order to detach the plug easily.

After almost one year of continuous operation the machine originally constructed is still in good operating condition. The only care it has received consisted of occasional cleaning and greasing.

CHEMICAL

A CLINICAL METHOD FOR THE DETERMINATION OF NICOTINIC ACID IN BLOOD AND URINE*

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IN A recent project undertaken to study the absorption rate of nicotinic acid, there was need for a simple, reliable method for its determination in reasonably small samples of blood. At the time there were two methods described, both depending on the color reaction between the product of cyanogen bromide treatment of nicotinic acid and aniline. This reaction (made more sensitive by a proper choice of coupling amine) is generally considered the most sensitive and specific test for nicotinic acid. Ritsert's[1] method was considered unsuitable for clinical and serial analysis since it involves drying of the blood with anhydrous sodium sulfate, continuous extraction with benzene, and evaporations before final color development. Pearson's[2] method utilizes 20 c.c. of blood and a tungstic acid precipitation. The large quantity of blood used, the necessity for very extensive evaporations, the use of an amine of low sensitivity (aniline) and failure to prepare a "blank" prevented the adoption of this method. There were also available two reports on the determination of nicotinic acid in natural products. The first by Bandier and Hald[3] employed p-aminophenol ("metol") as the coupling amine. The second by Harris and Raymond[4] reported a urine method using the still more sensitive p-aminoacetophenone and described in detail the conditions of hydrolysis necessary for the splitting of the naturally occurring nicotinic acid derivatives. In addition, a satisfactory method for determining the blank due to the action of the amine on other constituents of the urine was given.

From these various reports a satisfactory clinical method for determining blood nicotinic acid was developed. It employs an acid hydrolysis of a tungstic acid filtrate, using p-aminophenol as the coupling amine in acid phosphate solution. Although p-aminoacetophenone was found to yield more color per microgram of nicotinic acid, the color development was far more influenced by light and slight changes in the pH of the medium, than in the case of p-aminophenol. The latter, therefore, seemed more desirable for rapid and yet reliable analysis.

After the procedure was developed, two methods appeared in publication for nicotinic acid determination in blood. The method of Melnick and Field,[5] involving charcoal adsorption and aniline as the amine, did not seem well adapted

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to clinical use. That of Kodicek[6] employs p-aminoacetophenone and few enough operations to be an apparently satisfactory routine method.

The reliability and simplicity of the method to be described as tested by its application to approximately 250 blood specimens seemed to warrant a detailed report.

DETERMINATION

Apparatus and Reagents.—Any photoelectric colorimeter, or as was used in this laboratory, a Coleman "DM" photoelectric spectrophotometer.

10 per cent sodium tungstate and 2/3 N sulfuric acid.

2 per cent potassium dihydrogen phosphate, hydrochloric acid (36 per cent) and 10 N sodium hydroxide.

4 per cent aqueous cyanogen bromide, made fresh before each group of analyses. The solid material may be purchased from Eastman and keeps well in the icebox.

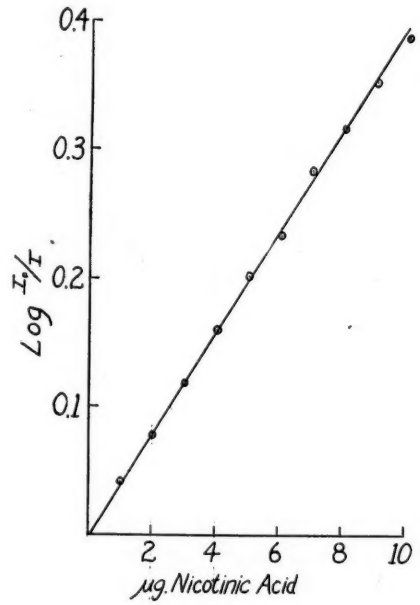

Fig. 1.

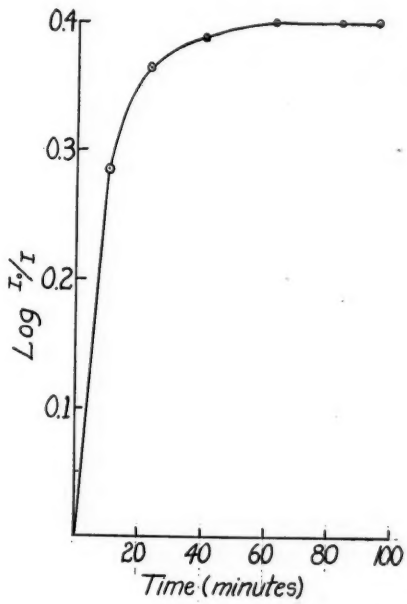

Fig. 2.

Fig. 1.—Linear relation between light absorption at $\lambda = 400$ mμ and amount of nicotinic acid. Colors were determined sixty minutes after start of color development.
Fig. 2.—Rate of color development in the dark using 10 μg of nicotinic acid.

Saturated p-aminophenol ("metol"). Dissolve approximately 5 Gm. in 100 c.c. of water, shake for five minutes, and filter. This must be made fresh before each series of analyses.

Method.—Five cubic centimeters of oxalated blood is hemolyzed in 35 c.c. of water, followed by 5 c.c. of 10 per cent sodium tungstate and with shaking 5 c.c. of 2/3 N sulfuric acid. After standing for ten minutes, it is centrifuged for fifteen minutes at 2,500 r.p.m. to obtain the maximum amount of filtrate. The supernatant fluid may be filtered through a moistened filter paper to eliminate any floating precipitate, and 30 c.c. of the clear filtrate is measured into a 50 c.c.

centrifuge tube bearing a mark at 10 c.c. The tube is immersed in boiling water and a strong jet of air is blown on the surface for rapid evaporation to at least the 10 c.c. mark. (In practice 4 or 6 tubes may be treated simultaneously, requiring twenty to twenty-five minutes for the operation.) Water is added to the 10 c.c. mark, and 1.6 c.c. of concentrated (36 per cent) hydrochloric acid is added and mixed. The tube is immersed in a boiling water bath for fifteen minutes, cooled, and neutralized to pH 5.0 with 10 N sodium hydroxide (approximately 2 c.c.) and with 0.5 N sodium hydroxide as pH 5.0 is approached. This can be done with sufficient accuracy by removing small samples with a glass rod and testing on Hydrion A* indicator paper. The tube is replaced in the boiling water bath and with the air stream is evaporated to the point where salt begins to crystallize out (approximately 2 c.c.). It is centrifuged briefly and the liquid is poured off into a test tube marked at 6.0 c.c. Two successive small additions of water are added, washing down the walls. After centrifugation the water is poured into the test tube to the 6.0 c.c. mark and the contents are mixed. This tube is labeled A, from which 2.0 c.c. portions are removed to tubes B and C. Tubes A and B each receive 2.5 c.c. of 2 per cent acid phosphate, and tube C receives 2.5 c.c. of 2 per cent acid phosphate containing exactly 4 μg of nicotinic acid. The tubes are placed in a water bath maintained at 75° to 80° C. Tube A receives 0.5 c.c. of water, tubes B and C, 0.5 c.c. of 4 per cent cyanogen bromide. After mixing they are allowed to remain for five minutes, cooled, and 5.0 c.c. of saturated p-aminophenol are added to each. The tubes are inverted for mixing and are placed in a dark closet for one hour, at which time they are ready for photoelectric measurement of the yellow color produced.

If the spectrophotometer is used, the contents of tube A are poured into the "solvent" tube, then the transmission of B and C successively measured at wave length $\lambda = 400$ mμ. In the case of the photoelectric colorimeter, a filter transmitting maximally at this wave length should be used. Since the color of C is due both to the unknown amount of nicotinic acid + 4 μg of nicotinic acid (reacting under identical conditions as the unknown), then the amount of nicotinic acid in the unknown can be expressed as:

$$U = \frac{\log {}^{I_0}/_{I_B}}{\log {}^{I_0}/_{I_C} - \log {}^{I_0}/_{I_B}} \times 4 \ \mu g$$

and in milligrams per cent of total nicotinic acid in the blood, 0.1 U.

In the case of the photoelectric colorimeters, the "zero" reading must be set with tube A, then the relations above are similar except that the log of the galvanometer readings are utilized.

The use of tube C is generally necessary in nicotinic acid analysis since salt concentration differences, pH, etc., may affect the amount of color obtained during the coupling reaction. In the case of blood analysis under these conditions it was found, however, that the same amount of color was regularly obtained from 4 μg of nicotinic acid in the presence of the prepared blood filtrate as in pure solution. In practice, therefore, the C tube was in-

*Distributed by R. P. Cargille, 118 Liberty Street, New York, N. Y.

cluded only occasionally. With our instrument and cells 4 μg of nicotinic acid gave a log = 0.160 ± 0.004. The useful range of the instrument employed includes values up to 1.8 mg. per cent (fasting level 0.34 to 0.45 mg. per cent). If higher values are expected, smaller samples should be used for analysis.

For nicotinic acid analysis in urine, 15 c.c. of the urine are mixed with 2.0 c.c. of 10 N sodium hydroxide and heated in a boiling water bath for thirty minutes. After cooling it is neutralized with concentrated hydrochloric acid to pH 5.0 and water is added to 20 c.c. Two cubic centimeters of this solution (one may expect from normal persons in the order of 5 μg per cubic centimeter of urine) or appropriate dilutions thereof are put into tubes A, B, and C, as described under Method.

In this case it is essential to determine for each urine or dilution, the "color yield" of a standard amount of nicotinic acid under the conditions used (difference between tubes B and C).

TABLE I

REPRODUCIBILITY OF BLOOD NICOTINIC ACID DETERMINATIONS AND RECOVERY EXPERIMENTS

SAMPLE	NICOTINIC ACID ADDED* (MG./100 C.C. BLOOD)	THEORETICAL (MG. %)	FOUND (MG. %)	RECOVERY (%)
1	--	--	0.36†	--
	--	--	0.34†	--
	0.40	0.75	0.71	–5.3
	0.60	0.95	0.92	–3.2
	0.80	1.15	1.18	+2.6
	1.00	1.35	1.34	–0.7
	1.50	1.85	1.74	–6.0
2	--	--	0.39†	--
	--	--	0.38†	--
	0.20	0.59	0.59	0.0
	0.50	0.89	0.85	–4.5
	0.90	1.29	1.31	+1.5
	1.50	1.89	1.86	–1.6

*In normal saline.
†Corrected for the dilution of the blood caused by adding saline.

The method here described is essentially a composite of several tested methods, designed for clinical use. The data collected in Figs. 1 and 2 and in Table I demonstrate that with the procedure described (a) a perfectly straight line relation was obtained between the log of the transmission at λ = 400 mμ, and the amount of nicotinic acid; (b) that in the development of the color the reaction is 95 per cent complete in forty minutes, and constant from fifty-five to ninety-five minutes standing in the dark; (c) that in duplicate analyses of a single specimen of blood, results are reproducible within 6 per cent; and (d) that in recovery experiments where the nicotinic acid (in normal saline) is added directly to the blood, never less than a 92 per cent recovery was obtained in the range 3.0 to 15.0 μg of nicotinic acid.

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A SIMPLE METHOD FOR THE DETERMINATION OF SERUM PROTEIN*

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THE Kjeldahl digestion method or one of its modifications is still the standard method for the determination of protein concentration in blood serum. The accuracy is adequate for most purposes, but the test is time-consuming. The clinical significance of the concentration of blood proteins has led to numerous investigations studying the significance of variations in blood protein level and methods of correcting hypoproteinemia (Bailey,[2] Allen,[1] Moon,[8] Shuman and Jeghers.[12] The search for simple, yet accurate, methods for determining blood protein concentration has, therefore, assumed importance.

The nephelometric method of Reiss[11] represents an early attempt at simplification. The technique is quick and easy to learn, but its accuracy, particularly in nephrosis and lipemia, has been seriously questioned by Linder, Lundsgaard, and Van Slyke,[7] and Guillaumin, Wahl, and Laurencin.[6] Fishberg and Dolin[5] suggested that the buffer action of blood is normally directly proportional to its protein concentration. They proposed a method of determining blood protein concentration, therefore, by measuring the resulting hydrogen-ion concentration of the blood after allowing it to react with a standard amount of acid. The results are reported to check very closely (within 0.5 per cent) with the Kjeldahl method. Its limitations are obvious in pathologic states in the presence of acidosis, alkalosis, or base depletion.

Moore and Van Slyke[9] demonstrated a close, practically linear, relationship between the specific gravity of blood and its protein concentration. They showed that the specific gravity of blood represented the protein level more closely than did its refractive index and was influenced less by variations in lipoid concentration. The specific gravity in their experiments was determined most conveniently by means of a specially constructed pyknometer. Barbour and Hamilton[3] had previously described a method for determining the specific gravity of blood by measuring its rate of fall through an immiscible fluid under standard conditions. Their technique was of sufficient accuracy to en-

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able detection of hemoconcentration in dogs after emotional excitement or injection of adrenaline, and of hemodilution after sectioning the splanchnic nerves. This method, with modifications, is being used extensively in clinical laboratories to determine the specific gravity of blood or serum and to compute therefrom the concentration of protein. The calculation depends upon the empiric formula [P = 343 (G – 1.0070)] of Moore and Van Slyke,[9] where P = concentration of serum proteins in grams per cent, and G = observed specific gravity.

Sufficient evidence has accumulated to show that the specific gravity is an accurate index of the protein concentration of blood and serum provided it can be determined with accuracy to the fourth decimal place. Both the pyknometer method of Moore and Van Slyke[9] and the falling-drop method of Barbour and Hamilton,[3] or its modifications, have been shown to be adequate. The former, however, requires experience with the pyknometer and the analytical balance. A volume of less than 2 c.c. of fluid to be tested impairs the accuracy of the method.

The falling-drop method is accurate and can be carried out with a single drop of blood or serum, but there are many possible sources of error and experience with relatively complex apparatus is required before determinations are dependable.

A modification of the specific gravity method for the determination of serum proteins has been devised and tested. It involves the use of calibrated hollow glass beads, each of a carefully predetermined density. This report embodies a description of the method and the results of experiments with it.

METHOD

Small beads (1.5 to 2 mm. in diameter) were blown from hard glass capillary tubing and flame-polished. They were made at random in large numbers and their densities were determined individually by matching them with carefully prepared solutions of potassium sulfate (K_2SO_4) of known specific gravity at room temperature. The bead which stayed wherever placed in the standard solution without rising or falling in thirty seconds was catalogued as representing that particular specific gravity. The values were checked at least twice. Twenty-one such beads were selected as representing specific gravities of 1.0350 to 1.0172 (or serum protein concentrations of 9.60 to 3.50). Beads can thus be made to represent any desired range of protein values.

To determine the specific gravity of an unknown fluid, a bead thought to approximate the unknown specific gravity is selected and with bone-tipped forceps is placed below the surface of the unknown fluid. If the bead rises, it is replaced by one of greater density, and vice versa if it falls. If the bead shows no motion during an observation period of thirty seconds, it is taken to represent the specific gravity of the fluid tested and its protein content, in the case of blood or serum, is calculated by means of the Moore and Van Slyke formula. The test can be done in small glass test tubes of 5 mm. diameter and any convenient height. It is possible to test 0.5 c.c. of blood or even less.

RESULTS

I. The accuracy of the method in determining the specific gravity of liquids was tested by comparing its results with those obtained by the use of the pyknometer described by Moore and Van Slyke. Table I represents a comparison of specific gravities obtained by the two methods for solutions prepared by adding varying amounts of powdered glucose to aqueous solutions or to blood serum. The close agreement of the two methods is evident, the maximum difference being 0.0009; the average, 0.00047, represents for serum a protein concentration of 0.16 gram per cent. It may be concluded, then, that the bead method for determining the specific gravity of serum is sufficiently accurate for clinical purposes.

TABLE I

SPECIFIC GRAVITY OF MODIFIED* SERUM AS DETERMINED BY THE GLASS BEAD AND THE MOORE AND VAN SLYKE PYKNOMETER† METHODS (Including Figures in Table IV)

NO.	BEAD	PYKNOMETER	DIFFERENCE BEAD-PYKNOMETER
1	1.0274	1.0278	–0.0004
2	1.0278	1.0280	–0.0002
3	1.0283	1.0292	–0.0009
4	1.0312	1.0308	+0.0004
5	1.0265	1.0261	+0.0004
6	1.0275	1.0270	+0.0005
7	1.0279	1.0276	+0.0003
8	1.0291	1.0283	+0.0008
9	1.0300	1.0293	+0.0007
10	1.0253	1.0254	–0.0001
11	1.0210	1.0212	–0.0002
12	1.0225	1.0226	–0.0001
13	1.0241	1.0244	–0.0003
14	1.0269	1.0276	–0.0007
15	1.0281	1.0289	–0.0008

Average difference in specific gravity = 0.00047 = 0.16 per cent protein. (Specific gravity 0.0001 = 0.0343 per cent protein.)

*Serum modified by adding solid glucose or distilled water.

†The pyknometer used here had a capacity of 10 c.c.

II. The serum protein concentration was determined in eighty-eight specimens of blood by the micro-Kjeldahl method and by the bead method described above. Fifteen more specimens were determined in duplicate. The thirty specimens were submitted for determination by both methods as unknowns.

Table II compares the results obtained by the micro-Kjeldahl and the bead specific gravity method in twenty representative determinations. The close agreement of the two methods is striking. The maximum difference between the results obtained by the bead and micro-Kjeldahl methods is 0.8 Gm. per cent. This difference appeared in only two of the 118 determinations. In two others the difference was 0.7 Gm. per cent, in one it was 0.6 Gm. per cent, and in nine it was 0.5 Gm. per cent. The average difference was 0.25 Gm. per cent.

Table III shows the results obtained in ten representative specimens out of fifteen which were determined as unknown duplicates by the two methods. It will be seen that closer checks, as a rule, are obtained by the bead method than by the micro-Kjeldahl digestion method.

TABLE II

SERUM PROTEIN CONCENTRATIONS (GRAMS PER CENT) IN TWENTY REPRESENTATIVE SPECIMENS DETERMINED BY THE MICRO-KJELDAHL AND THE GLASS BEAD METHODS

NO.	MICRO-KJELDAHL	GLASS BEAD	NO.	MICRO-KJELDAHL	GLASS BEAD
1	6.1	5.7	11	2.7*	<3.5†
2	4.3	4.7	12	4.5*	3.7
3	6.4	6.4	13	6.6	6.0
4	5.5	5.4	14	4.9	5.2
5	7.6	7.5	15	7.6	7.8
6	5.0	4.8	16	6.8	6.0
7	9.6	8.9	17	4.3	3.8
8	4.1	4.3	18	8.0	7.9
9	7.0	7.0	19	3.7*	3.9
10	4.4*	4.5	20	3.6*	3.7

*From patients with nephrosis.
†This represented the lightest bead in the set.

TABLE III

SERUM PROTEIN CONCENTRATION (IN UNKNOWN DUPLICATES) DETERMINED BY THE MICRO-KJELDAHL AND THE GLASS BEAD METHODS

NO.	MICRO-KJELDAHL	GLASS BEAD
1	6.1	6.4
	6.4	6.4
2	6.3	6.0
	6.6	6.0
3	5.2	5.6
	5.7	5.6
4	5.2	5.4
	5.5	5.6
5	5.4	5.6
	5.6	5.6
6	6.4	6.4
	6.8	6.4
7	4.8	5.5
	5.2	5.7
8	6.8	6.5
	6.8	6.6
9	7.6	7.5
	7.6	7.5
10	5.5	5.7
	5.5	5.7

TABLE IV

EFFECT OF VARYING THE GLUCOSE CONCENTRATION ON THE SPECIFIC GRAVITY OF BLOOD SERUM

GLUCOSE CONCENTRATION (MG. %)	SPECIFIC GRAVITY		SERUM PROTEIN DIFFERENCE	
	BEAD	PYKNOMETER	BEAD	PYKNOMETER
100	1.0265	1.0261	0	0
200	1.0267		0.07	
330	1.0275	1.0270	0.34	0.31
500	1.0278		0.44	
570	1.0279	1.0276	0.48	0.52
790	1.0291	1.0283	0.89	0.76
1,050	1.0300	1.0293	1.20	1.10

III. *The effect of glucose concentration on the specific gravity of blood serum.* The glucose concentration of normal blood serum was varied by the addition of powdered glucose and the specific gravity of the solution was determined by the bead method in seven instances. In five of these, it was determined also by the pyknometer method. The results are shown in Table IV and graphically in Fig. 1, in which the specific gravity difference is converted to the serum protein

concentration difference in grams per cent. Reference to these data demonstrates a practically linear error in the estimated serum protein concentration introduced by variations in the glucose concentration of the blood serum. This observation is of significance clinically for blood protein determinations done in patients with diabetes mellitus.

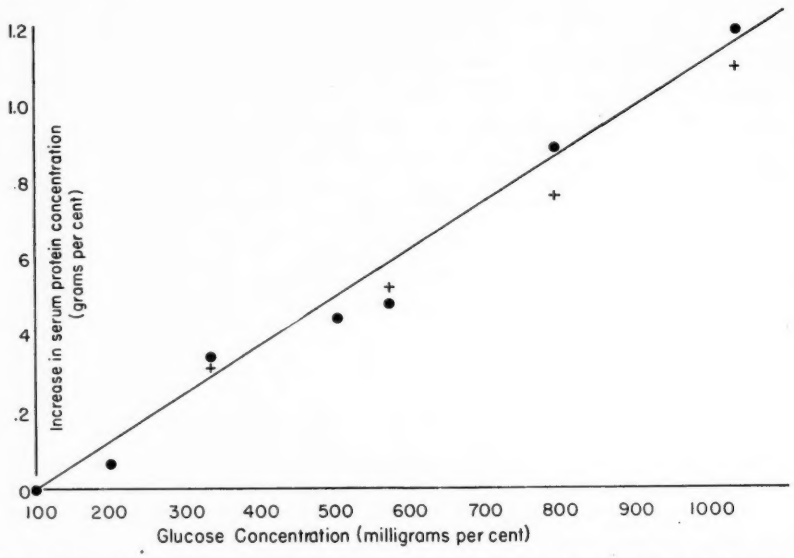

Fig. 1.—Graph relating glucose concentration of serum to specific gravity. Ordinates: specific gravity recorded as protein concentration; abscissae: glucose concentration; crosses: values obtained by pyknometer method; dots: values obtained by glass bead method.

DISCUSSION

The foregoing data demonstrate the practicability of a method for the determination of serum proteins, depending upon the virtually linear relationship between specific gravity and protein concentration. The specific gravity is accurately measured by means of hollow glass beads of previously determined density. Bing[4] described a specific gravity method for determining serum protein by the use of a single bead representing a specific gravity of 1.0158 (protein concentration of 3.0 Gm. per 100 c.c.). Three cubic centimeters of serum are used and diluted with 0.9 per cent sodium chloride until the bead neither rises nor falls. At room temperature (20° C.) the diluted volume represents the protein concentration of the serum. The accuracy of the method depends in part upon the accurate delivery of 3.0 c.c. of serum. An error of 0.1 c.c. will introduce an error of 3.4 per cent in the total protein concentration. If smaller volumes of serum are used, the error will be correspondingly greater. It is convenient in that it involves the use of only one bead. This advantage over the method described here loses significance when it is considered that once a set of beads is prepared, it can be used indefinitely and the necessity of volumetric instruments and diluting fluid is obviated.

The accuracy of any method depending upon the relationship between specific gravity and serum protein concentration could be influenced by a number of factors. The effect of changes in the albumin-globulin ratio was studied

by Nugent and Towle.[10] They observed no significant effect of varying the albumin-globulin ratio of beef serum. Increases in the lipoid concentrations likewise have relatively little influence on the specific gravity of serum (Moore and Van Slyke). The slight shifts that take place in the concentration of blood solids when the protein level changes have been found to be of no practical significance (Weech, Reeves, and Goettsch[13]). The data in Table IV show that abnormal concentrations of blood glucose may introduce a significant error in the protein concentration computed from the specific gravity. Sufficiently accurate corrections may be made by deducting 0.1 Gm. per cent protein for each increment of 100 mg. per cent glucose.

Certain precautions should be observed in determining specific gravity by the method described here. The beads are best kept in a padded partitioned box and handled with bone-tipped forceps. The blood or serum is placed in small glass tubes held vertically in a plasticine base. Determinations are done at room temperature. To avoid surface tension effects, the bead is placed in the solution directly below the surface. An air bubble may be carried down with the bead and should be carefully shaken off when present. Currents are sometimes set up in the fluid by the mere introduction of the forceps into it, particularly when tubes of larger diameter are used. If the bead is observed for thirty seconds, however, the effect of currents will have been spent. The beads, of course, should be kept clean, and this is easily accomplished by rinsing them in distilled water immediately after removing them from the blood or serum.

The method for blood protein determination presented in this report is offered as a quick, simple, yet accurate, one. No contention is held that this method is more accurate than the other specific gravity methods or the Kjeldahl method, but, since the technique is simple and sources of error are minimized, it will find greater adaptability in clinical research. For ordinary clinical purposes it is hardly necessary to have more than four calibrated beads representing serum protein concentrations of 5.0, 6.0, 7.0, and 8.0, respectively. With experience, it becomes simple to interpolate between beads and read values to within 0.25 Gm. per cent. Greater accuracy in the clinic is seldom required.

SUMMARY

1. A method for the estimation of blood or serum protein concentration is described, depending upon the determination of the specific gravity of the fluid by means of calibrated hollow glass beads.

2. The method compares favorably in accuracy with other reported techniques. Its advantages are its sensitivity, its simplicity, and its requirement of very small volumes.

3. The effect of glucose concentration on the determinations has been tested and a graph is presented (Fig. 1) from which corrections may be made.

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NOTE ON A POSSIBLE SOURCE OF ERROR IN THE DETERMINATION OF SULFAPYRIDINE AND SULFANILAMIDE*

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THE twofold purpose of this paper is (1) to point out a possible source of error in the determination of sulfanilamide and sulfapyridine, and (2) to indicate that concentrations of procaine hydrochloride as low as 0.01 mg. per cubic centimeter of blood may be determined with a high degree of accuracy, thus making available a method for the study of the metabolism of procaine hydrochloride.

In the course of a series of sulfapyridine determinations on a patient to whom sulfapyridine was administered parenterally at a constant rate, it was observed that there was a rather marked fluctuation in the blood level of sulfapyridine. The fluctuation in blood level did not correspond to alterations in the dose or rate of administration of sulfapyridine. It was apparent, therefore, that some contaminant might possibly be responsible for the observed fluctuations. Upon inquiry it was found that it has been common practice for hospital interns to collect venous blood in syringes which had been used to anesthetize the skin, with 2 per cent novocaine (procaine hydrochloride), prior to venipuncture.

It should be noted that the color development of sulfapyridine and sulfanilamide depends upon diazotization of primary aryl amines. Procaine hydrochloride is a primary aryl amine and diazotization of its amine group, when the sulfapyridine reagents are added, according to the method of Marshall,[1] results in a pink color which is identical in tinctorial power with that obtained

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from sulfapyridine. As an indication of the magnitude of this source of error, it was found that after one drop of 2 per cent procaine hydrochloride is added to 5 c.c. of blood, a sulfapyridine value of 12.3 mg. per cent was found. There is no significant diminution in the value if the blood is allowed to stand at room temperature for two days.

An attempt was made to recover added procaine hydrochloride from blood to determine the lower limits of sensitivity of the method. Accordingly, procaine hydrochloride was added to blood in amounts sufficient to yield final concentrations of 1.0 and 2.0 mg. per cent. Precipitation of the blood was then carried out in 1:10 and 1:20 dilutions. The recovery of procaine hydrochloride from the blood containing 1.0 mg. per 100 c.c. was 1.02 mg. per cent in the 1:10 dilution of the blood, and 1.04 mg. per 100 c.c. in the 1:20 dilution of the blood, an error of plus 2 and plus 4 per cent. Recovery of procaine hydrochloride from blood containing 2.0 mg. per cent with 1:20 dilution was 2.14 mg. per cent, an error of plus 7 per cent. In these determinations the blood estimation was matched with a known procaine hydrochloride standard.

TABLE I

STANDARD SODIUM SULFAPYRIDINE MOL. WT. 271 (MG. %)	STANDARD SULFANILAMIDE MOL. WT. 172 (MG. %)	STANDARD PROCAINE HCl MOL. WT. 272 (MG. %)	DETERMINED PROCAINE HCl (MG. %)	CALCULATED PROCAINE HCl CONCENTRATION (MG. %)
–	0.20	0.20	0.130	$0.13 \times \frac{272}{172} = 0.20$
–	0.40	0.40	0.256	$0.256 \times \frac{272}{172} = 0.40$
0.20	–	0.20	0.200	$0.200 \times \frac{272}{271} = 0.20$
0.40	–	0.40	0.396	$0.396 \times \frac{272}{271} = 0.39$

It now seemed desirable to determine the tinctorial power of procaine hydrochloride as compared to sodium sulfapyridine and sulfanilamide. Standard 0.2 per cent and 0.4 per cent solutions of these substances were prepared. As may be seen in Table I, the color developed by procaine hydrochloride is practically identical with that developed by sulfapyridine—the molecular weights are nearly identical. When procaine hydrochloride is matched with standard sulfanilamide, procaine hydrochloride only develops about 64 per cent as much color. However, the ratio of the molecular weights of procaine hydrochloride and sulfanilamide is $\frac{172}{272} = 1.58$, indicating a 100 per cent recovery on an equimolecular basis. It is apparent then that on an equimolecular basis the tinctorial power of procaine hydrochloride is equal to that of sulfanilamide and sulfapyridine.

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OBSERVATIONS WITH A PHOTOELECTRIC HEMOGLOBINOMETER*

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PHOTOELECTRIC colorimeters have been adapted to many measurements, including the quantitative estimation of hemoglobin. Reported observations indicate the feasibility of rapid and fairly precise hemoglobin measurements by the use of such instruments.[1-6] The present study was undertaken to survey the characteristics of a photometer designed particularly for use as a hemoglobinometer.

THE INSTRUMENT

The instrument used was a simple form of photoelectric colorimeter,† consisting essentially of a single photoelectric cell, a filter, a holder for solution containers, and a light source. The photoelectric cell was of the blocking layer type, the output being measured with a sensitive galvanometer built into the instrument. The lamp was energized by a pair of dry cells incorporated in the instrument case. The solution containers were commercially obtainable test tubes selected for uniformity in size of bore. The holder for the solution containers was of special construction to compensate for optical defects inherent in a cylindrical form of solution cell. Fig. 1 shows the general appearance of the instrument.

The apparatus was operated as follows: With the lamp off, the galvanometer needle was adjusted to a prescribed zero point at the right extremity of the scale by manipulating a small knob mounted on top of the instrument (Fig. 1). The lamp was then lighted, and a solution cell containing freshly distilled water was placed in the holder. This procedure caused movement of the galvanometer needle to the left. The needle was then adjusted to the left extremity of the scale by altering the lamp intensity by means of a rheostat provided on the front of the instrument (Fig. 1). The tube containing distilled water was then replaced by one containing an unknown solution of acid hematin prepared as noted below. The decreased transmission of light caused a deflection of the galvanometer needle to the right for a distance dependent upon the concentration of acid hematin present in the solution. For most consistent results the instrument was allowed to stand with the lamp lighted for about five minutes prior to the first reading. Before use tubes for solutions were carefully cleansed inside and out.

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†This photometer was an experimental model supplied by the Fisher Scientific Company, Pittsburgh, Pa.

EXPERIMENTAL PROCEDURE AND RESULTS

Five samples of beef blood, one of sheep blood, and two of human blood were examined. Upon each of these the following observations were made:

a. Oxygen capacity by the manometric method of Van Slyke and Neill,[7] carried out in duplicate or triplicate.

b. Oxygen capacity by the blood iron method of Wong.[8]

c. Photometer readings, after appropriate treatment, of whole blood and of dilutions with 0.9 per cent sodium chloride solution containing 80, 60, 40, and 20 per cent whole blood.

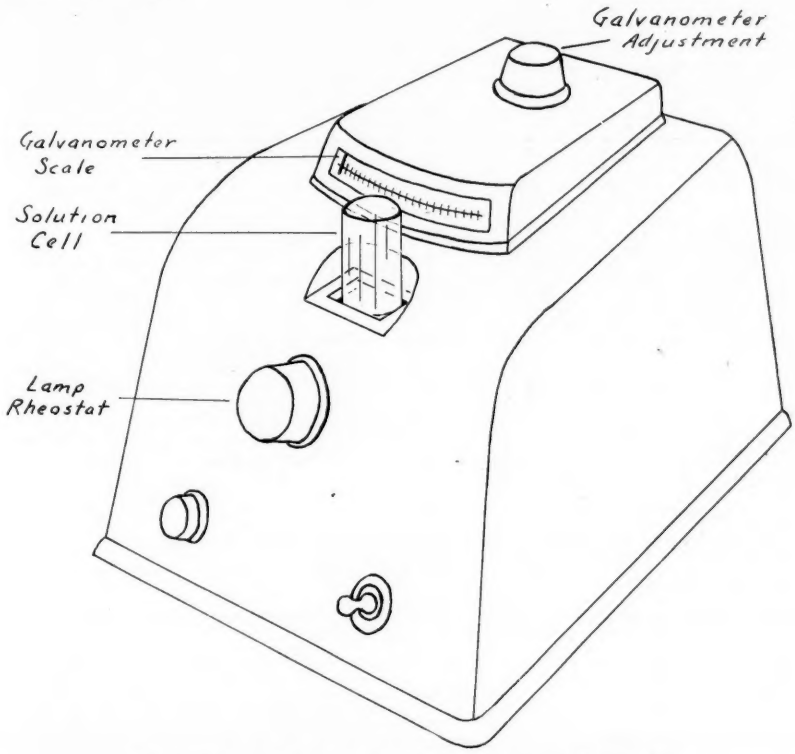

Fig. 1.—General view of photoelectric hemoglobinometer.

Samples of whole or diluted blood were prepared for photometric studies by diluting in a special pipette 20 c.mm. of the sample with 5 c.c. of tenth-normal hydrochloric acid. The resulting acid hematin solution was discharged into a solution cell and allowed to stand for five minutes. Although the color was not fully developed in this length of time, results were sufficiently consistent for practical purposes. Three or more photometer readings were made for each test, the galvanometer zero being reset, if necessary, between readings.

Table I shows the relationship of hemoglobin content, calculated from oxygen capacity, to photometer readings expressed in arbitrary units of the galvanometer scale. This relationship is shown graphically in Fig. 2, which is based on hemoglobin values obtained by the gasometric method. In the preparation of Fig. 2 hemoglobin contents of blood dilutions were calculated from the values determined for respective samples of whole blood.

TABLE I

RELATIONSHIP OF THE HEMOGLOBIN CONTENT OF BLOOD TO PHOTOMETER READINGS

BLOOD NO.	NATURE OF BLOOD	O_2 CAPACITY		HEMOGLOBIN		PHOTOMETER READING UNITS
		GASOMETRIC (VOL. %)	BLOOD IRON (VOL. %)	GASOMETRIC (GM. %)	BLOOD IRON (GM. %)	
2	Sheep	15.45	16.40	11.6	12.3	36.0
3	Beef	16.49	16.87	12.4	12.7	35.3
4	Beef	21.31	22.10	16.0	16.6	40.2
5	Human	11.48	13.20*	8.6	9.9*	30.0
6	Human	8.97	10.12*	6.7	7.6*	25.8
7	Beef	19.99	20.50	15.0	15.4	38.6
8	Beef	22.66	23.25	17.0	17.5	42.2
9	Beef	22.29	22.47	16.7	16.9	41.5

*Patients supplying these samples were receiving iron medication daily.

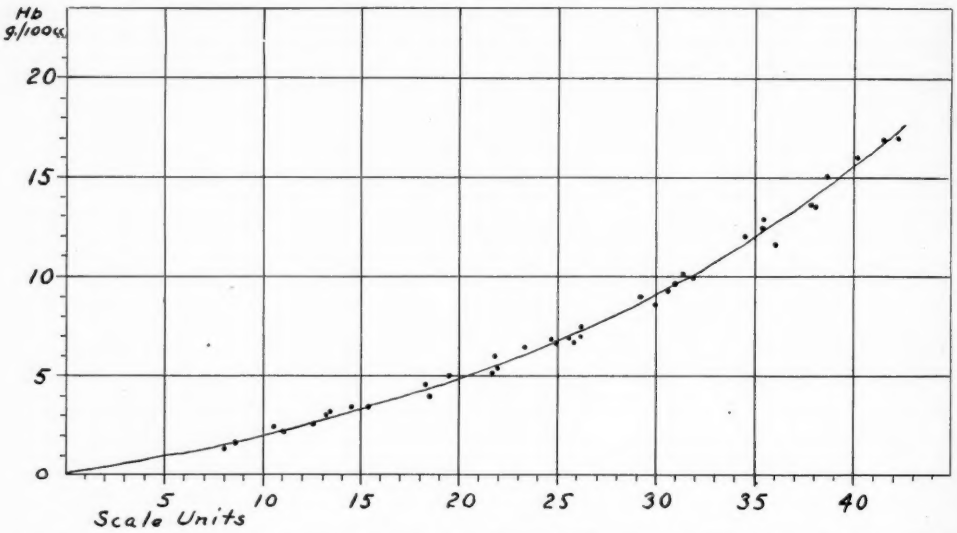

Fig. 2.—Relationship of photometer readings to hemoglobin concentration, based on gasometric hemoglobin determination.

DISCUSSION

The characteristics of this particular type of photometer favored its application to hemoglobin estimations. Operation was simple, requiring less than ten minutes for a complete determination. The amount of blood required was small and the final dilution was high, conditions which tend to minimize the effects of technical errors in sampling and dilution. As shown in Fig. 2, the calibration curve was quite flat, especially in the clinically important low hemoglobin range. Single readings were reliable within one unit on the galvanometer scale in approximately 90 per cent of consecutive trials. A deviation of one scale unit was equivalent to 0.3 Gm. of hemoglobin per 100 c.c. of blood below 5 Gm. total, 0.3 to 0.5 Gm. between 5 and 10 Gm. total, and 0.5 to 0.7 Gm. between 10 and 15 Gm. total hemoglobin content. Repetition of photometer readings was but a matter of seconds and naturally reduced the error of single readings.

A point of incidental interest was the comparison of hemoglobin measurements by gasometric and blood iron methods, as shown in Table I. The iron

method gave results consistently higher than those obtained by gasometric determination, the average difference amounting to 0.61 Gm. of hemoglobin per 100 c.c. of blood. The widest discrepancies occurred in the two human samples, both from anemic patients receiving large daily doses of iron. If these two samples are neglected, the average difference between results by the two methods was 0.45 Gm. per 100 c.c. We are inclined to agree with Haden[9] that either method is suitable for normal bloods, and that the relative simplicity of the iron method is a definite advantage. However, it seems clear that the true respiratory value of the blood is more accurately portrayed by the gasometric measurement.

SUMMARY

1. The characteristics of a simplified form of photoelectric hemoglobinometer were studied by comparison of photometer readings with hemoglobin measurements according to the gasometric method of Van Slyke and Neill and the blood iron method of Wong.

2. The photometer employed was found to be suitable for hemoglobin measurements.

3. Single readings were reliable within 0.3 to 0.7 Gm. of hemoglobin per 100 c.c., depending on the total hemoglobin content of the sample. Multiple readings, occupying an insignificant amount of extra time, narrowed the limits of error.

4. Comparison of hemoglobin estimations by gasometric and blood iron methods was included in the study.

It is a pleasure to acknowledge the helpful suggestions of Dr. C. C. Guthrie.

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A TECHNIQUE FOR THE DETERMINATION OF PROTHROMBIN TIME*

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WITH the introduction of the effective use of vitamin K in the treatment of hemorrhage in jaundice by Warner, Brinkhous, Smith[1]; Butt, Snell, Osterberg[2]; Dam and Glavind,[3] the need for a practical and accurate method of determining the prothrombin content of the blood, now known to be related to vitamin K absorption, has assumed increasing importance.

The techniques for prothrombin determination have been limited to two accepted methods: that of Dam and Glavind[4]; which is extremely time-consuming and laborious; and that of Quick, which is more practical for clinical purposes, and the more widely used of the two. Even the method of Quick,[5] however, is burdensome, since it does not include a stable thromboplastin, and therefore requires frequent renewal of the preparation.

During the past year a technique has been developed and successfully employed in our laboratories which eliminates the above-mentioned disadvantages of time and complexity, and so facilitates the increased use of prothrombin determination, that we feel it merits a detailed description.

The apparatus employed consists of a constant temperature water bath, set at 40° C., and an anatomic museum jar, measuring 25 by 21 by 9 cm., with sides of fairly uniform thickness, as the water tank. The heat source is a knife-blade heater connected to a mercury thermoregulator by means of a magnetic relay. The water between the heater and thermoregulator is kept in motion by a stirrer attached to the shaft of a slow motor, as illustrated in Fig. 1.

The reagents required are a potent thromboplastin extract and a 1/40 M calcium chloride solution. The thromboplastin is obtained by extraction from the brains of stillborn fetuses. To obtain consistent results with extracts prepared by this method, from a number of brains, the brain should not be kept in the refrigerator for more than thirty-six hours after death. To prepare the brain for extraction, the pial membranes and vessels are removed as completely as possible, and the brain is cleaned by immersing for a moment in cold water. It is then weighed, cut into small pieces, and put into a large mortar. An equal weight of pure sand is added and the mixture is ground with a pestle until it has an even creamlike consistency. To this mixture is added an approximately equal volume of normal saline and thoroughly mixed. The material is then centrifuged until the supernatant fluid is only slightly cloudy. This takes approximately one to one and a half hours. The fluid is then poured off, filtered through cotton, sealed in 5 c.c. vials, and kept frozen by storage in a freezing chamber. Thromboplastin prepared from a single brain and stored in

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this manner has been kept for six months without any signs of deterioration in potency, and has been sufficient in quantity for approximately 200 determinations.

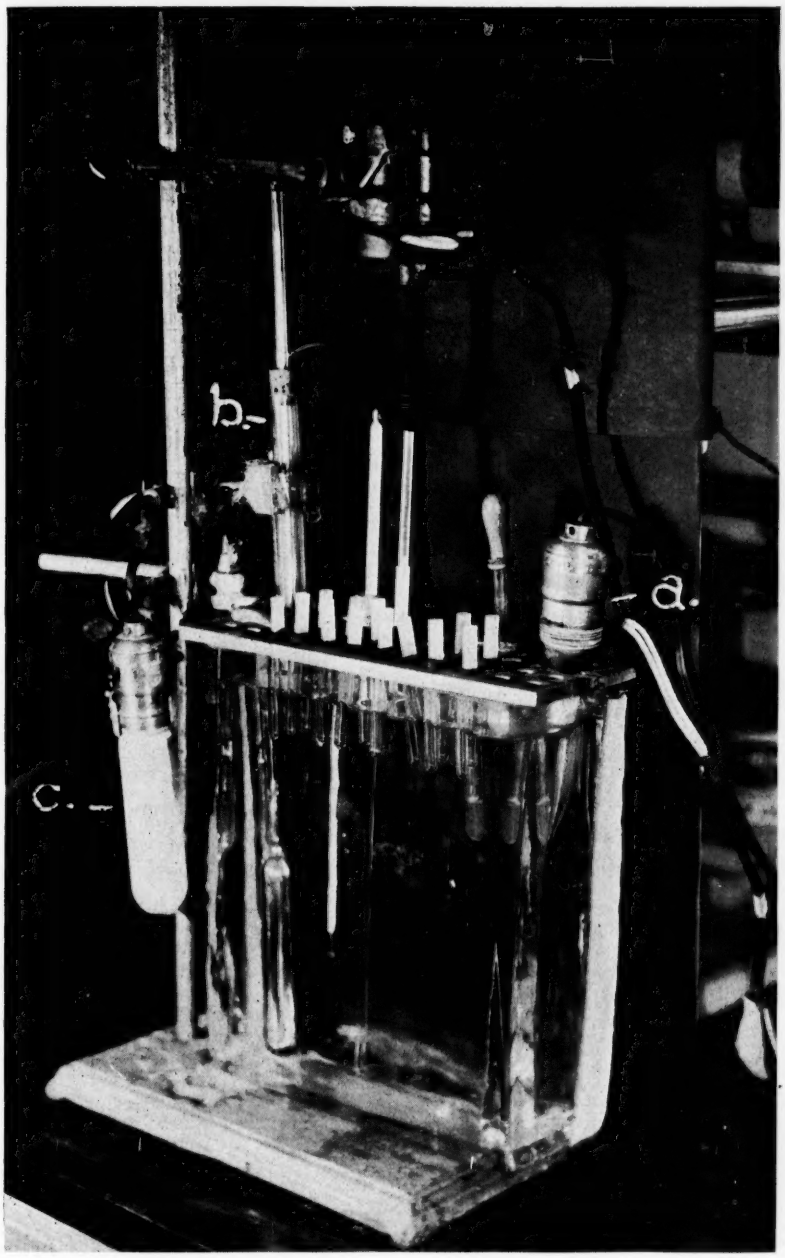

Fig. 1.—*a*, Knife blade heater; *b*, mercury thermoregulator; *c*, light source.

In preparation for the test a vial of the thromboplastin extract is defrosted in warm water, and an equal volume of 1/40 M calcium chloride solution is added to it. This mixture is then immersed in the water bath, which is kept at

40° C., and allowed to incubate for thirty to forty-five minutes, after which time it will be noted that a flaky white material has been precipitated, and the supernatant fluid is almost clear. This fluid is centrifuged at high speed (about 2,000 r.p.m.) for ten minutes until clear, and the clear fluid is then returned to the water bath.

Fig. 2.—*a*, Ground glass surface for labeling.

Fig. 3.

To collect the blood sample, 15 c.c. calibrated centrifuge tubes, containing 0.5 c.c. of 3 per cent sodium citrate solution, are used, to which 5.5 c.c. of blood are added, giving a total of 6 c.c. The plasma and cells are separated by centrifuging at 2,000 r.p.m. for five minutes, as soon as received, the plasma being kept in the refrigerator until used for the determination. Blood collected and treated in this fashion will yield a plasma which will give reliable results even after twenty-four hours, in contrast to the thirty minutes previously considered safe.

To determine the prothrombin time, 0.1 c.c. of plasma is pipetted into special round bottom tubes (Fig. 2), which in the meantime have been allowed to come to the temperature of the water bath. The plasma is incubated in the water bath for five minutes. Approximately 0.2 c.c. of the calcium chloride thromboplastin mixture is drawn up into the aspirator (Fig. 3) and is quickly expelled into the tube containing the plasma. At the same time the stop watch is started. The tube is viewed at right angles to the source of illumination, and when clotting appears, manifested by the beginning of opacity, the watch is stopped. (The reading of opacity is greatly facilitated by painting the back of the water bath black.) The time noted is the prothrombin time. By this method normal human plasma has a range of 10 to 13.3 seconds and an average of 12.1, with a mean variation of 0.7 second. With but little experience one can repeat readings on a given sample of plasma within 0.6 second. For routine work three readings are done; these should agree with each other within the limits of one second.

Recently I have used a thromboplastin extract prepared from brains as those described, but have varied the method somewhat to eliminate the necessity for adding calcium chloride at the time of determination. In this case, the extraction medium is a solution of 1/40 M calcium chloride in normal saline. A mixture of these two substances is centrifuged and stored in vials as described above. If, when doing the determination with this extract, one desires a greater volume of thromboplastin extract, addition of an equal volume of normal saline will not diminish its potency. With this preparation it is important that the extract incubate in the water bath for forty-five minutes before centrifuging. Results with this extract on normal plasma are somewhat higher than those described. By this method normal human plasma has a range of 15.3 to 24.9 seconds and an average of 20.8, with a mean deviation of 1.9 seconds. This extract has proved as stable and reliable as the one originally used.

Experiments with a thromboplastin extract in which an equal volume of 1/40 M calcium chloride solution was added to the thromboplastin after its extraction with normal saline and then stored as mentioned above, yielded an extract similar in potency to the one extracted with 1/40 M calcium chloride in normal saline. This preparation, however, showed evidence of deterioration after two months. The use of stillborn fetus brains has yielded a thromboplastin superior in activity and stability to that extracted from the brains of embryo and adult cattle and rabbits, as well as adult human beings.

SUMMARY

1. The preparation of a stable and potent thromboplastin extract which eliminates the repeated extraction of thromboplastic material is described.

2. A description of a simple, inexpensive, and reliable technique and apparatus for the determination of prothrombin time is presented.

Sincere thanks are extended to Mr. Bernard Sobel, of the Division of Chemistry, for his aid in assembling the apparatus.

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THE IMPORTANCE OF TEMPERATURE FOR COLORIMETRIC DETERMINATIONS*

With Special Reference to Nessler's Reaction

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COLOR reactions for determining biological constituents are widely used in this country. In most cases the colored solutions to be compared display a more or less colloidal behavior.

A few incidental observations showed that for one of these colorimetric methods, the yellow color produced by adding Nessler's reagent to ammonium sulfate solutions obtained by digesting urine with sulfuric acid, is very dependent upon the temperature.

As shown by many investigators the color produced by Nessler's reagent in solutions of ammonia goes parallel to the amount of nitrogen whether the ammonia was obtained by distillation or the nitrogen solution digested by sulfuric acid was immediately used for the test (direct nesslerization).

To avoid complications resulting from the digesting of organic material in the way commonly used for nitrogen determinations, the following experiments were made with pure ammonium sulfate solutions.

Into each of two large test tubes graduated to 35 and 50 c.c., 5 c.c. of ammonium sulfate solution containing 1 mg. of nitrogen were pipetted, 1 c.c. of sulfuric acid solution (1 c.c. of concentrated sulfuric acid, 1 c.c. of water) added and made up to 35 c.c. with water. The tubes were kept in ice water for fifteen minutes. Nessler's reagent just taken from the refrigerator was slowly added to the mark 50, mixed, and the tubes left in the ice water for three more minutes. After this time one tube was left in the ice water, the other was taken out and kept at room temperature.

Both cups of the colorimeter were filled with the solution in ice water. Readings were in the beginning 30:30; after being left in the cups ten minutes, 30:29.7; after twenty minutes, 30:29.6; after thirty minutes, 30:29.7. Thus the readings were practically constant for the solutions, ice cold in the beginning, being warmed together to room temperature by standing in the cups.

The solution (now at room temperature) was left in one of the cups and the solution in the other cup replaced by new solution kept at room temperature, but the reading did not change, 30:29.5, 30:29.8. Thus with both solutions at the same temperature the reading is correct and stays that way.

When the room-warm solution was left in one cup and cold solution (from the test tube in ice water) was substituted in the other cup, the reading was

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30:17.5 (30 being the layer of the ice-cold solution). While the solutions were in the cup, the temperature of the cold solution slowly went up, the reading went down to 30:24, and after thirty minutes to 30:29, practically reaching the correct reading.

If the cups were emptied and one cup was filled with ice-cold solution and the other with solution at room temperature, the reading was 30:17.5 and went down to 30:25, and finally to 30:30, when the solutions had reached the same temperature. These experiments were duplicated in manifold ways. Parts of the same nesslerized solution showed the phenomenon, if one-half was kept cool, the other half at room temperature, and the difference in reading could almost be reversed by reversing the temperature of the solutions. The changes of the optical transmission of these solutions were hardly visible to the naked eye; the solutions looked clear and transparent. It may be added that there was no difference whether a protective colloid was added or not.

The explanation of these findings is, of course, given by the colloidal status of the solutions, the optical transmission being decreased by raising, and increased by lowering the temperature. As far as can be seen in these few experiments this process is, at least for a certain time, more or less reversible. Theoretical questions cannot be discussed in this paper.

Some other color reactions, such as the determination of phosphorus with hydroquinone-molybdic acid (Bell and Doisy) and the determination of glucose with the Benedict method did not show this influence of temperature.

Using Nessler's method, whether with or without preceding distillation and with or without protecting colloid, care must be taken that the temperature of the solutions in the cups is the same. For practical purposes the best procedure seems to be to add cold Nessler's reagent to the cold solutions to be compared, add if desired the protective colloid, mix, leave the solutions in the cold for ten minutes, take them out and let them stand for twenty minutes at room temperature. In this way the solutions have the same temperature and are aged to the same degree. For each reading not only the solution to be tested but also the standard solution may be put in the cups at the same time, thus avoiding the danger of unequal warming by the light source.

MEDICAL ILLUSTRATION

CLINICAL LABORATORY PHOTOGRAPHY WITH A NEW METHOD OF PREPARING PHOTOMICROGRAPHS*

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THE value of a simple and inexpensive method of routine photography for use in the modern clinical laboratory need not be emphasized. Indeed, there are few institutions possessing aggressive staffs which have not recognized the value of such facilities and attempted a solution. Few hospitals that are not directly associated with teaching institutions or research laboratories have the services of trained photographers for their requirements. The responsibility is usually relegated to the pathologist or radiologist who far too frequently has not had the opportunity for study or experience in this field. As a result, the prospect of starting such a department is alarming from the triple viewpoint of expense, time involved, and results produced.

For those laboratories and institutions already possessing cameras and equipment suitable for gross and photomicrographic work, the methods outlined here may be of little value. There is no denying the fact that better photographs are easier to obtain by using more expensive precision apparatus and larger film sizes or plates; however, it is possible to work with 35 mm. film and obtain excellent results. It might be found advantageous in terms of simplicity and economy to consider the procedure given here for routine photomicrographic work. Hellwig[1] has stressed the need for such procedures and given a method. To the best of my knowledge there has been no prior publication dealing with photomicrographs by the method here described, yet from its very simplicity it must have been used.

GROSS AND CLINICAL PHOTOGRAPHY

Equipment.—The equipment listed here is for the convenience of those desiring such information. There are many other similar cameras and apparatus available which undoubtedly will do equally as satisfactory work. The following equipment can be purchased for less than one hundred dollars.

1. Argus C_2 35 mm. camera, or for those desiring photoflash equipment the C_3 is available at a slight additional cost.

2. The Arguscope, which essentially gives accurate ground-glass focusing for close work.

3. Photoelectric cell type light meter.

*From the Laird Memorial Hospital, Department of Pathology, Montgomery.
Received for publication October 10, 1940.

4. Any 35 mm. film enlarger. A considerable saving can be effected by using an enlarger that will accommodate the camera lens.

Technique.—For photographing patients, equipment, and large specimens, or for use in the necropsy room, the camera alone is satisfactory. It is equipped with a coupled range finder, and accurate focusing from three feet can be done easily.

The Arguscope is invaluable in photographing surgical, necropsy, or other small objects. Full instructions are supplied by the manufacturers and are extremely simple. Photographs may be taken covering fields from 8½ by 12¾ inches down to 1 by 1½ inches, and at distances from 19½ inches down to 3¾ inches from the object. The use of the ground-glass attachment for critical focusing and composition enables the routine production of prints of excellent quality.

Mallory[2] gives directions for preparing a tank devised by Bitterman for photographing specimens. The use of a tank is satisfactory, although in using 35 mm. film a smaller tank, that is, one that encompasses a field equal to the area covered by the camera, is also satisfactory. The purpose of photographing specimens under water is to eliminate annoying high lights. A defect that develops from lighting the specimen while under water is flatness or a lack of modeling. It is advisable, therefore, that one of the lights be placed at an acute angle to produce modeling. A far better method of eliminating high lights and still preserving the modeling is to light the specimen through a tracing cloth screen as described by Schmidt and Haulenbeek.[3]

The background of gross specimens may be made white on the print by using an auxiliary light beneath a glass base upon which the specimen may rest. The same thing can be accomplished by opaquing the background in the negative. However, the former method is easier and better. A more complete discussion of this subject will be found in another publication.[4]

The use of a celluloid ruler provides an easy method for critical focusing and in addition gives an easily interpreted scale for determining the size of the specimen from the prepared photographs. The ruler should be placed one-third distance from the top of the specimen, or two-thirds distance from the background. After focusing on the ruler, the diaphragm is closed as far as possible to obtain the maximum depth of focus. When the specimen is placed on a clear glass background with a light beneath, a transparent celluloid ruler is most effective.

Film, including the 35 mm. size, is available in many different types of emulsions. I have found the Eastman Panatomic X, processed in the Eastman DK-20 developer, to be the most satisfactory for general use in clinical and gross specimen photography. This 35 mm. film may be purchased in handy cartridges already prepared by the manufacturer, although a considerable saving may be effected by the use of bulk film. The almost routine photographing of surgical specimens and the attaching of prints to the completed reports necessitate development of the negatives fairly soon after their exposure. By purchasing film in bulk quantities and loading old cartridges with film lengths between twelve and fifteen inches, one is able to obtain eight to ten exposures for

each strip, instead of waiting several days as is often necessary to complete the exposures on a long roll of film. The DK-20 developer can be used for a considerable number of such strips without appreciable deterioration.

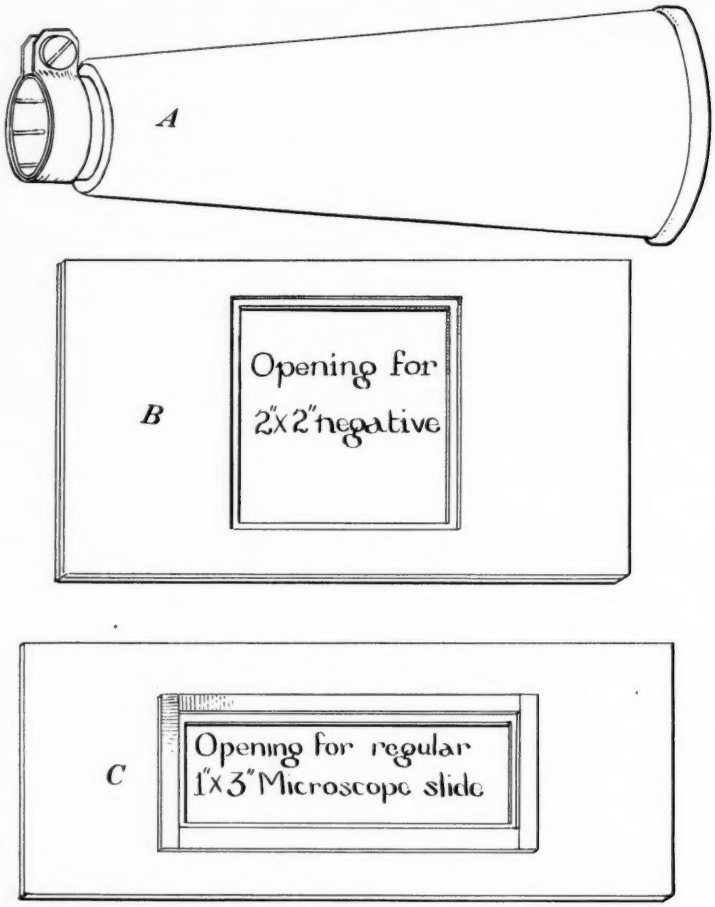

Fig. 1.—*A*, Cone-shaped adapter for fitting on microscope to hold the lantern slide. *B*, Cardboard enlarger carrier to hold 2 by 2 inch negative. *C*, Enlarger to hold 1 by 3 inch microscope slide.

Miniature camera work is extremely well adapted to the use of color film, and Kodachrome transparencies (type A, indoor) add greatly to the value of the work performed. For viewing these transparencies a regular 2 by 2 inch projector is available, or the larger-sized projectors may be converted by a simple attachment to handle the 2 by 2 inch sizes. In the event these adapters are used on the large projectors, it is advisable also to change the lens of the large projector to one of a shorter, proper focal length. In this manner the image on the screen will be the same size as the image projected from the larger slide, provided the projector is at the same distance from the screen. In changing back to the larger slide it becomes necessary to change the lenses again.

An attractive method of displaying these 2 by 2 inch transparencies, and likewise keeping them, has been developed by the Filmdex Corporation of Mt. Kisco, New York.

Extremely satisfactory colored photomicrographs for projection or use as transparencies for museum purposes can be prepared very simply from black-and-white lantern slides by the method described by Putnam.[5] This method is also applicable to the preparation of transparencies by means of Eastman Translite enlarging paper.

PHOTOMICROGRAPHS

The Arguscope can be used in photomicrographic work if desired. The results are variable; however, the method given here has proved satisfactory.

The equipment required is minimal, provided one possesses a microscope and a lamp. Beyond these, a darkroom, the adapter to be described, and a supply of 2 by 2 inch medium lantern slides are all that are necessary. The adapter consists of a cone (Fig. 1*A*) which fits over the monocular tube and accommodates the ocular. The tube shown in the illustration measures 5½ inches in total length and 1⅞ inches in diameter at the top. However, these tubes can be made in different lengths according to the additional magnification or reduction that is desired in relation to the objective and ocular used. By doubling the length of the tube the magnification is increased about one-fourth. Of course, if a bellows is used in place of the tube, the image can be varied to fill out the film with a specific composition. The exact magnification can then be determined by means of a stage micrometer. The inside of the adapter or bellows is painted dull black to prevent light reflections. These adapters are easily prepared or can be obtained from H. F. Holmes, Montgomery, W. Va., for two dollars. A bellows and the necessary supports would be more expensive.

In use the adapter is fitted to the microscope, as shown in Fig. 2, and the top of the cone is covered by a piece of ground glass, measuring 2 by 2 inches, with the ground surface down. This is easily prepared by removing the emulsion from a 2 by 2 inch lantern slide and then rubbing briskly with a valve grinding compound, procurable at any garage, until an evenly ground surface is obtained. The placing of penciled cross lines in the center enables one to focus accurately on the ground surface.

The lamp is tilted or twisted until the beam of light from the condensing lens falls squarely on the center of the microscope mirror. The condensing lens in the best lamps for photomicrography is adjustable, so that the beam can form a small spot on the mirror. The smaller this spot the more intense is the light, thus making short exposures at high magnifications possible. Lamps that are more suitable for photomicrography are the ribbon filament, the arc, the Point-o-lite, and the new photomicrographic lamp of Bausch & Lomb Optical Co. and General Electric Co. The arc is the most common in use, but the ribbon filament lamp is rapidly taking its place. The Point-o-lite is an excellent light source but somewhat difficult to use. The new photomicrographic light is not yet in common use and its value, therefore, cannot be judged. In any event, the beam of light is centered accurately, the condenser of the microscope is brought into focus, and the objective filled with light; these procedures are necessary in all photomicrographic work and are accomplished easily. A slide is inserted and the microscope is brought into focus on the ground glass; the focus is checked by means of a hand lens. The field desired to be reproduced

is selected; obviously, it should be free from defects or artifacts. The microscope lamp is turned off, the ground glass is removed, and a 2 by 2 inch film or plate is put in place.

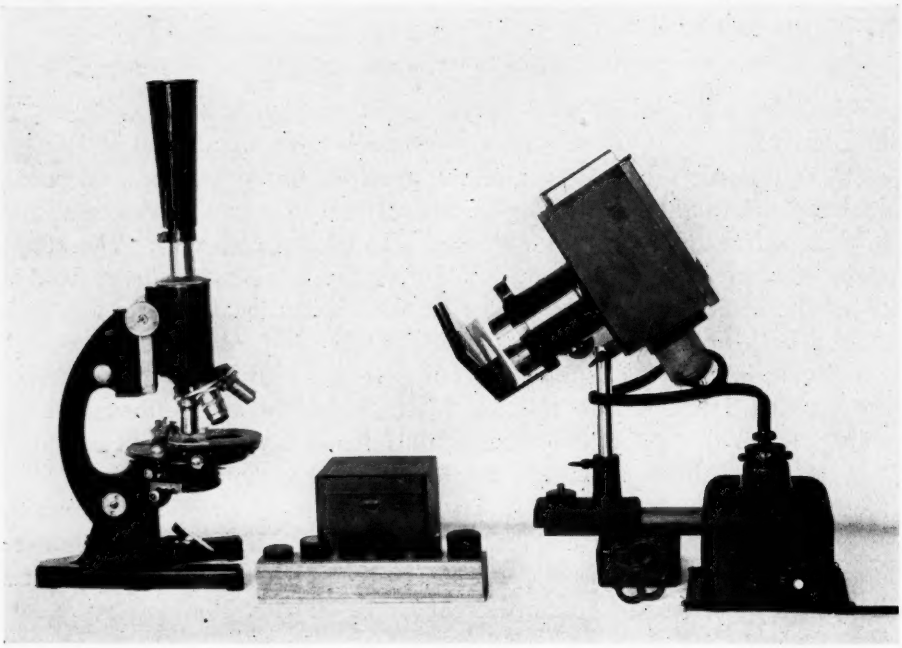

Fig. 2.—Microscope showing the adapter in place. In this picture the ribbon filament 6 volt lamp is used. This lamp has a condensing lens for spotting the light on the mirror of the microscope and a holder for two filters which are seen in place. Other light sources are discussed in the text. The lenses used in making the pictures seen in Fig. 3 are also shown. The box is for holding the various color filters.

In considering the sensitized negative materials most suitable for photomicrography, one should choose the type of film or plate on the basis of contrast desired and its sensitivity to the colors to be recorded. Most workers find it desirable to use a plate of high available contrast, high resolving power, and general sensitivity to all colors. The Wratten M plate for all types of photomicrography that do not require extreme sensitiveness is generally used. This plate, if properly employed, will produce negatives of a wide range of contrast, depending on the factors of varying the exposure and developing time. Photographic plates having only the original color sensitivity of the silver salts to ultraviolet, violet, and blue lights are technically considered to be "ordinary" plates (commercial and process), and may be developed in red light. When the sensitivity is extended by dyes into the green, the plates are orthochromatic. These, too, may be developed in a red light of low intensity. Panchromatic materials are sensitive throughout the whole visible spectrum and sometimes even beyond, into the near infrared.

With panchromatic material it is generally necessary to use a light filter between the light source and the condensing system of the microscope. It is best to select the desirable filter by visually inspecting the subject through filters until one is found that shows up the subject to best advantage. No one filter will fit all cases, nor can the filter be disregarded in every instance in

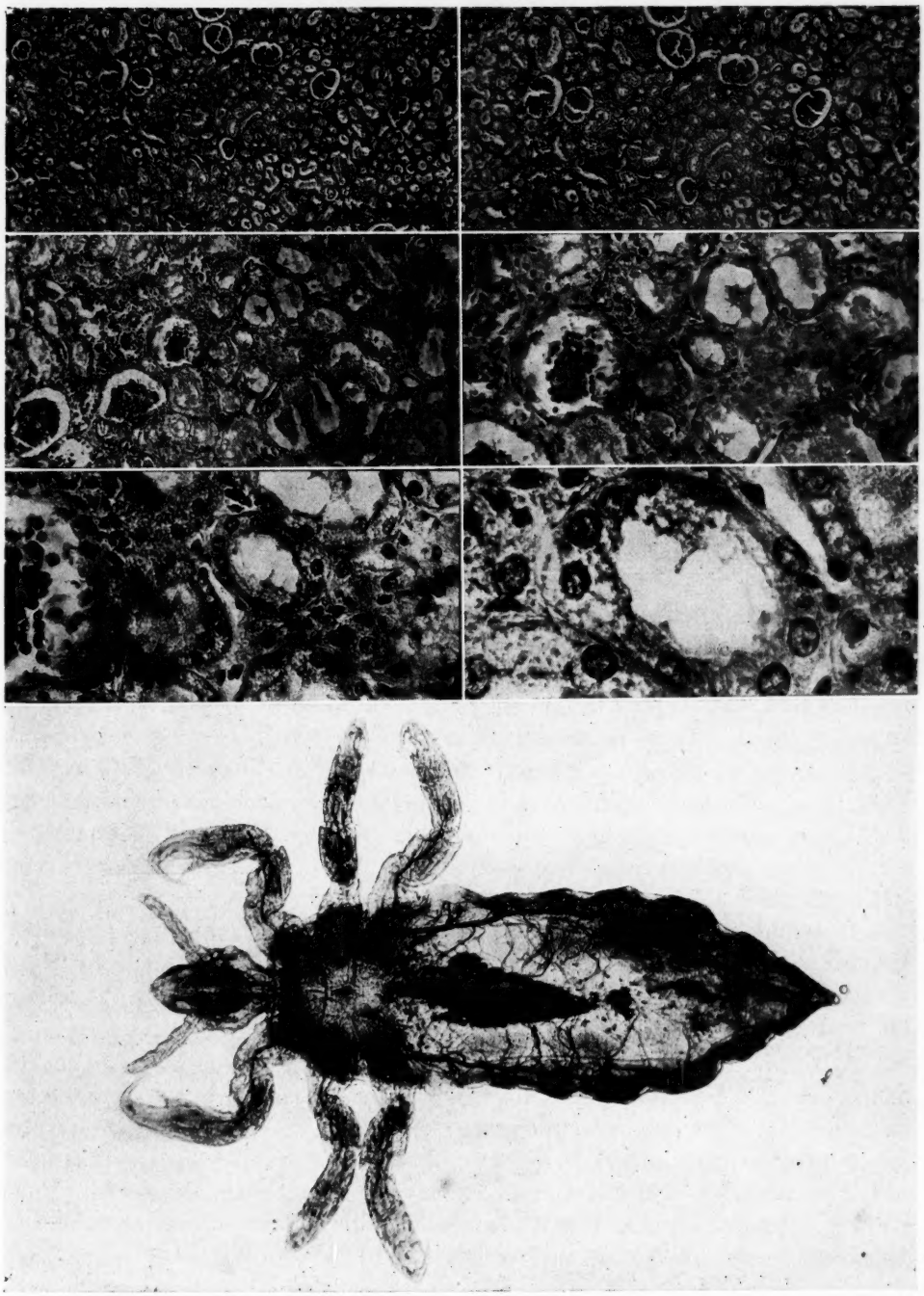

Fig. 3.—Photomicrographs showing six different magnifications of the same area of kidney tissue. A × 7 ocular was used on each photomicrograph. The magnifications of the objectives were as follows: *A*, Micro Tessar series, 32 mm.; *B*, × 5 Apochromat; *C*, × 10 Apochromat; *D*, × 20 Apochromat; *E*, × 40 Apochromat; *F*, × 90 Apochromat; *G*, A typical low power photomicrograph of a parasite.

spite of the fact that a process or process panchromatic plate will at times give the desired contrast and detail in the resulting negative.

The book *Photomicrography*[6] gives the following table which is of assistance in choosing a filter suitable for specific cases:

Use for blue stained preparations a red filter
Use for green stained preparations a red filter
Use for red stained preparations a green filter
Use for yellow stained preparations a blue filter
Use for brown stained preparations a blue filter
Use for purple stained preparations a green filter
Use for violet stained preparations a yellow filter

In some cases it is necessary to use two filters and panchromatic material to obtain the best results. For example, a green and a yellow filter in combination often show up the hematoxylin-eosin stained section most satisfactorily.

All the foregoing photographic work can be done in a darkroom, provided the plate or film is not incased in a holder or magazine. Exposure of the film can be made by snapping the microscope light on and off; only a few trials are needed before one can accurately judge the correct exposure. Development is carried out in total darkness with the time and temperature method if panchromatic material is used; otherwise the ruby light is employed and some control over density is possible. It is also permissible to use lantern slide plates for making the negatives. These plates are very contrasty and correspond to a process emulsion. Furthermore, they are considered to be "color blind" in the sense they do not give true color values in black and white. This finished plate is a negative and may be used by contact printing to produce a print or, better yet, an enlargement. These enlargements are easily made by cutting a cardboard or plywood carrier to fit in place of the usual 35 mm. film carrier (Fig. 1*B*). There is a minimum of grain present in the lantern slide emulsion and enlargements of a number of diameters are possible (Fig. 3). Lantern slide positives may be prepared by contact printing on 2 by 2 inch slides, or by enlargement on the standard 3¼ by 4 inch lantern slides.

In taking photomicrographs by the method discussed, the size of the field encompassed is limited. With this in mind experiments were made to find a practical solution for getting around this difficulty. It was solved in the following manner:

A 35 mm. enlarger was used with a cardboard carrier cut so as to enclose in the insertion a standard glass slide, 1 by 3 inches, on which was mounted the tissue section. One can insert the carrier with the slide in the enlarger and project the entire image of the section on a 2 by 2 inch or a 3¼ by 4 inch lantern slide as a negative. No filters are necessary for an approximate record. However, if panchromatic negative materials and filters are used over the enlarger lens, better color renderings and contrasts will be obtained. Of course, such work must be done in total darkness, or while using a green safelight. The negative in either case should be sharp; by using it as outlined excellent prints may be obtained. This approximates very low power photomicrography in an extremely simple manner.

Photomicrographic negatives have been prepared on lantern slide plates from sections stained with hematoxylin-eosin, polychrome methylene blue, and

Masson's trichrome stain. Each of these methods of staining reproduces satisfactorily, and the use of panchromatic materials and filters has not been found necessary in all cases. In blood films, Wright and Strumia's stains have been used with excellent results.

GENERAL

An extremely interesting and relatively simple method for the coloring of photographs of gross specimens is given by Hopkins.[7] A red to red-brown color on prints is obtained as follows: solution A, 45 grains of uranium nitrate and 10 ounces of water; solution B, 40 grains of potassium ferricyanide and 10 ounces of water. Equal volumes of each solution are mixed, and 20 minims of glacial acetic acid are then added to each ounce of the mixture. Prints should be prepared in the usual manner, and thoroughly washed and dried. Artist's gum mastic is applied to all areas in which it is desirous of obtaining red. The gum mastic dries rapidly. The print is washed in several changes of distilled water and toned in the solution with the depth of color visually controlled. The prints are then blotted and dried, after which the gum mastic may be removed with alcohol. A satisfactory blue may be added by covering all parts of the print except those which are desired to take the blue color. A solution consisting of the following ingredients is prepared: ½ ounce of 10 per cent solution ferric ammonium, ½ ounce of 10 per cent solution potassium ferricyanide, and 5 ounces of 10 per cent solution acetic acid. The print is immersed until it assumes a dark greenish-blue color, after which it is washed thoroughly. The blue color is intensified by fixing in a hypo bath, and the gum mastic is then removed with alcohol. This method, though somewhat time-consuming and formidable at first glance, is surprisingly easy after a few trials, and the results are well worth the time expended. This subject is discussed more fully in another article.[8]

SUMMARY

Procedures suitable for routine photography in a clinical laboratory are discussed, and certain technical details are given. A method of preparing photomicrographs is outlined. Its value lies in the simple equipment necessary, the foolproof technique, and the quality of photomicrographs obtained. Attention is called to methods for preparing lantern slides of histologic specimens in color and for preparing prints of gross specimens in color that are suitable for use in one's own laboratory.

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DEPARTMENT OF ABSTRACTS AND REVIEWS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

TYPHOID CARRIERS: Soluble Iodophthalein in Treatment of Carriers of Typhoid-Paratyphoid Group, Saphir, W., and Howell, K. M. J. A. M. A. **114**: 1988, 1940.

A series of conservative measures, including the use of sulfanilamide, failed to render the stools of a carrier of *Bacterium paratyphosum A* free from these bacilli.

The oral administration of soluble iodophthalein was followed by immediate disappearance of the paratyphoid A bacilli from the stool. Repeated stool examinations extending over a seven months' observation period remained consistently negative for these organisms.

The results of a bacteriologic study in vitro as to the bactericidal action of soluble iodophthalein on the bacilli of the paratyphoid group did not entirely explain the curative action in a human carrier.

SULPHANILAMIDE, Antienzymic Nature of Bacteriostatic Action of, Mellon, R. R., Locke, A. P., and Shinn, L. E. Am. J. M. Sc. **199**: 749, 1940.

The free amino group of sulfanilamide when incompletely oxidized becomes poisonous for catalase, as a result of which the toxic hydrogen peroxide produced by many pathogenic organisms is permitted to accumulate in their cultures.

In addition to catalase, other enzymes are adversely affected by the intermediate oxidation products of the sulfonamide compounds. As known so far, they are peroxidase, certain dehydrogenases, and nitrotase. The nutrition of the bacteria is thus seriously affected, and bacteriostasis results.

The effect of such bacteriostasis against pneumococci in vivo is to bring about their "dissociation" into culture phases, whose principal metabolic functions, including hydrogen peroxide formation and virulence, are so critically suppressed that the organisms fall easy prey to the destructive action of the phagocytes.

BRUCELLA, Studies on a Purified Antigen From, Morales-Otero, P., and Gonzalez, L. M. Am. J. M. Sc. **199**: 810, 1940.

A method for the preparation of a purified protein derivative from brucella cells is described. The product obtained is of a fine consistency, light brown in color, and of a fairly constant chemical composition; it can be measured accurately and constitutes a good antigen.

The preparation has been used successfully as an antigen in complement fixation tests and in determining cutaneous hypersensitiveness to brucella in man and laboratory animals.

In man there is a direct relationship between contact with infected material or ingestion of infected raw products and skin reaction to purified brucella protein.

The incidence of positive reactors to the skin test in Puerto Rico, where *Br. abortus* infection is prevalent in cattle, was 24.7 per cent in contact groups, and 4.2 per cent in groups from the general population consuming raw milk from infected sources. On the island of St. Thomas, where infection is not known to exist, the authors were unable to find any reactors in the group examined.

The method follows: Large amounts of *Br. abortus* are grown on standard nutrient agar pH 7.8 in Blake bottles for seventy-two hours at 37° C. Approximately 5 ml. of saline with 0.5 per cent phenol are added to each bottle and left for thirty minutes in contact with the culture to loosen the cells. The phenolized saline containing the suspension of cells is removed from the bottles with sterile pipettes, and the surface of the agar is thoroughly

washed with more phenolized saline. The organisms are recovered by centrifuging at high speed, and dried in a calcium chloride desiccator until they attain a horny appearance. Then they are ground in an electric grinding apparatus to obtain a fine white powder. This fine powder is mixed with 2 liters of 0.02 N sodium carbonate and placed in the refrigerator overnight. Next morning the alkaline suspension is filtered, first through a paper and then through a Berkefeld candle. To the filtrate is added 0.5 per cent phenol, to avoid bactetrial contamination during the following steps of the procedure. The filtrate is then concentrated by ultrafiltration through alundum cups impregnated with 13 per cent gum-cotton glacial acetic solution, prepared according to the method described by Seibert. The ultrafiltration is continued until the liquid is concentrated to about 100 ml., then it is washed by passing 500 ml. of distilled water through the alundum cup filter. The concentrated colloidal solution is then filtered through a paper to eliminate any insoluble material that may have sedimented during the ultrafiltration. The protein is precipitated by mixing the colloidal solution with one-fourth its volume of a 50 per cent freshly prepared aqueous solution of trichloracetic acid, making a final concentration of 10 per cent acid. After standing overnight in the icebox, the protein precipitate is thrown down by centrifugation. Then it is washed repeatedly by centrifugation with freshly prepared 10 per cent trichloracetic acid solution until the washings are colorless. The protein product is then washed with large volumes of anhydrous ether by repeated triturations and centrifugations until all the acid is removed and the precipitate is completely dehydrated. The nitrogen and polysaccharide contents of protein prepared from different batches of cell cultures vary between 1 and 2 per cent.

The final product thus obtained fulfills the requirements, for it is of a fine consistency, light brown in color, and of a fairly constant chemical composition; it can be measured accurately, is stable in the dry state, and constitutes a good antigen. It is not completely soluble in water, but is easily dissolved by adding a few drops of 0.1 N alkali. The solution is neutralized with 0.1 N hydrochloric acid and remains clear. This preparation has been used in determining cutaneous hypersensitiveness among contact groups and groups from the general population of the island of Puerto Rico, where endemic abortion is prevalent, and in the island of St. Thomas, where endemic abortion is not known to exist. At the same time, agglutinative, complement fixation, and opsonocytophagic tests were made on the same subjects.

GLUCOSE TOLERANCE, Effect of Large Doses of Insulin on, Appel, J. W., and Hughes, J. Am. J. M. Sc. **199:** 829, 1940.

The authors recognize the great value of the glucose tolerance test as usually performed. However, the examination of the urine during the three days following the test appears to be even more useful in determining the actual carbohydrate tolerance. In the cases reported the usual tests would have indicated either more insulin or less carbohydrate, though further examinations as described showed that neither step was indicated.

Two of the cases examined over several weeks showed subjective and objective clinical improvement. Thus it would seem that the authors' procedure could be employed to advantage where more carbohydrate is desirable but more insulin is contraindicated. A simple glucose tolerance test without blood sugar estimations but with recordings of the twenty-four-hour urinary glucose over a period of several days before and after the test will indicate whether a given case can utilize additional carbohydrate.

The authors believe that many diabetic persons would benefit from the procedure herein described.

TUBERCLE BACILLI, Effect of Egg Oil on, Steenken, W., Jr. Am. Rev. Tuberc. 42: 422, 1940.

The experiments reported demonstrate that the tubercle bacillus will propagate in egg oil and that the resulting colonies will be smooth and creamy. They also show that this smooth appearance will be manifested by both dissociated variants and by undissociated cultures, and that the resulting colonies cannot be differentiated from one another.

These smooth colonies retain their altered appearance only while they are growing in the presence of the egg oil. Since this occurs regardless of the pH of the medium, the process cannot be a true dissociation; the smooth form of the colony is merely a physical effect of the oil upon its contours.

BLOOD, A New Test for, and Its Application to the Micro-Determination of Hemoglobin, Barrett, J. F. Brit. J. Exper. Path. **21:** 22, 1940.

The reduced form of the oxidation reduction indicator 2, 6-dichlorophenol indophenol is a useful reagent for the detection of blood. In the presence of hydrogen peroxide and blood pigment a red color is produced. A response is obtained with a 1:50,000 dilution of blood.

The reaction has been utilized to determine the hemoglobin content of minute quantities of blood. The equivalent of 0.00025 ml. is required for a determination.

The method for hemoglobin determination follows:

Reagents.—(a) 0.2 per cent alcoholic hydrogen peroxide. Add 0.5 ml. of 30 per cent hydrogen peroxide (perhydrol) to 75 ml. alcohol.

(b) Reduced indicator solution. Mix 0.2 Gm. of 2, 6-dichlorophenol indophenol with 120 ml. of glacial acetic acid. Add 25 ml. of 1 per cent stannous chloride solution ($SnCl_2.2H_2O$) in 20 per cent acetic acid. Stir until the dye dissolves, warming slightly if the dye is granular. Finally add 20 Gm. of crystalline sodium acetate dissolved in 50 ml. of water. Store the reagent in a dark stoppered bottle.

(c) Stock hemoglobin solution. Determine the hemoglobin content of a sample of normal blood by the oxygen capacity method of Van Slyke and Neill (1924). Dilute the blood with 25 per cent alcohol to give a solution of hemoglobin containing 1.3 Gm. per 100 ml. Store the solution in a stoppered bottle in the refrigerator.

Test for Blood.—Place 5 ml. of the specimen in a clean test tube, add 2 ml. of glacial acetic acid and boil the mixture thoroughly. Cool the tube to room temperature and add 5 ml. of ether. Extract the blood pigment by inverting the tube about twelve times. Place 5 to 10 drops of reduced indicator solution in a second test tube, and add 2 to 3 ml. of alcoholic hydrogen peroxide. Transfer 1 to 2 ml. of ethereal extract from the first test tube to the mixture of reduced dye and hydrogen peroxide in the second test tube. A rose-red color develops in the presence of blood. If no color appears in two minutes, the test is negative.

Microdetermination of Hemoglobin.—Dilute the well-mixed specimen of blood 2,000 times with water, using a carefully calibrated pipette. Prepare a working standard solution of hemoglobin by diluting the stock 1.3 per cent hemoglobin solution 200 times with water, e.g., 0.5 ml. to 100 ml.

Pipette 2 ml. of the reduced indicator solution into two test tubes labeled "standard" and "unknown." Add 2 ml. of the alcoholic hydrogen peroxide and mix thoroughly, making sure that no hydrogen peroxide is left on the sides. Add 0.5 ml. of the standard and immediately afterwards 0.5 ml. of the unknown solution of hemoglobin to the appropriate tubes and mix thoroughly. After one hour add 10 ml. of water to each tube, inverting several times to mix the contents. Compare the unknown with the standard in the colorimeter, using a green glass light filter to facilitate the matching.

$$\text{Calculation: } \frac{\text{Reading of Standard}}{\text{Reading of Unknown}} \times 13 = \text{Grams of hemoglobin per 100 ml. blood.}$$

When a blood low in hemoglobin is encountered, a more suitable dilution, e.g., 1:1,000 or 1:500, should be made.

LEUKOCYTOSIS, On the Mechanism of, With Inflammation, Menkin, V. Am. J. Path. **16:** 13, 1940.

Leukotaxine, a crystalline nitrogenous substance recovered from inflammatory exudates, and known to increase capillary permeability and cause diapedesis of polymorphonuclear leucocytes, fails to induce an increase in the leucocytic level of the circulation in either the dog or the rabbit. Hence it can scarcely be responsible for the leucocytosis of inflammation.

There is present in exudates a leucocytosis-promoting factor capable of inducing a rise in the leucocyte level of dogs. This is demonstrable when either the whole or the cell-free exudate is introduced into the circulating blood.

An inflammatory exudate removed from an animal with leucopenia and injected into a normal dog fails, as a rule, to induce a marked rise in the leucocyte count.

Blood serum, sterile broth, bacterial cultures of exudates, and cultures of killed bacteria *(Staphylococcus aureus)* are all ineffective in causing an increase in the leucocyte level of the circulation.

Histamine and adenosine are likewise essentially inactive. Extraction of exudates containing the active leucocytosis-promoting factor for histamine, yields an end product incapable of inducing leucocytosis. Nucleic acid injected into the blood stream in large concentration favors, after a considerable latent period, a rise in the number of leucocytes. This delayed effect differs from the prompt response induced by exudates.

The action of the leucocytosis-promoting factor seems to be primarily on the bone marrow, producing an outpouring of immature granulocytes into the circulation. The factor is thermolabile. Heating the exudate to 60° C. inactivates the material. The factor, after prolonged dialysis of the exudate through a cellophane membrane, is found to be, in large part, indiffusible. Further studies are now in progress in an endeavor to identify the nature of the leucocytosis-promoting factor present in inflammatory exudates.

PNEUMONIA in Infants and Children, Pneumococcus Typing in, Bullowa, J. G. M., and Simon, H. Am. J. Dis. Child. **60**: 256, 1940.

With the method of pharyngeal evacuation applied to a mummied infant and accomplished by the use of a suction bulb with catheter and trap, pulmonary mucus obtained for typing pneumococci gave a type in 95 per cent of cases. The portable apparatus for evacuation is simple to prepare and use.

The apparatus consists of a No. 18 French rubber catheter, with an opening at the end. The catheter is attached to a fire-polished and annealed pyrex glass trap about 4 inches (10.16 cm.) long. From the glass trap through pressure rubber tubing, the catheter is attached by brass tubing to a sputum bottle stoppered with a No. 5 rubber stopper containing two holes. The outlet from the sputum bottle, also of brass, leads to a double action rubber bulb or to an electric suction pump. The pump is of the rotary compressor type, operated by a $\frac{1}{30}$ horse power universal motor.

INFECTION, A Ward Study for Sources of, Long, A. P., McKhann, C. F., and Cheney, L. L. Am. J. Dis. Child. **60**: 322, 1940.

A direct, concurrent study of hospital infections in an infants' hospital confirmed the findings derived from an earlier survey of the records of the same institution.

Infections of the respiratory tract accounted for 63 per cent of the hospital infections which were acquired by over 10 per cent of the patients admitted. Most of the secondary infections occurred in very young, premature, malnourished, or debilitated infants who had been in the hospital for several weeks.

Acute infections of the respiratory tract, including pneumonia, present among the patients on admission as well as similar infections and carrier states among the staff, attendants, and visitors, were the great source of danger.

Examinations for new nasopharyngeal bacterial invaders in secondarily infected patients indicated the source of most of the infections to be hospital personnel and visitors rather than fellow patients.

Overcrowding in connection with hospital-acquired infections should be considered, with the term "overcrowding" applied not only to patients but also to the large numbers of persons other than patients who are permitted to enter the wards.

Some diminution in the transmission of organisms followed masking of all persons entering the ward. Masking, even with a special type of mask, was insufficient to eliminate secondary infections.

TISSUE: Rapid Method of Staining Gram-Positive Organisms in Frozen Sections, Krajian, A. Arch. Path. 30: 614, 1940.

Method.—

1. Fix tissues in a 10 per cent solution of formaldehyde for twenty-four hours or longer.
2. Cut thin frozen sections.
3. Transfer the section to a glass slide.
4. Drain and dehydrate immediately with absolute alcohol or anhydrous isopropanol. Blow on the section until all the alcohol is evaporated.
5. Blot dry.
6. Dip twice in thin celloidin (a pyroxylin preparation). Blow on the section and dip in water.
7. Stain with Sterling's gentian violet for two minutes.
8. Rinse in water and apply Gram's iodine solution for two minutes.
9. Blot completely dry.
10. Flood the section with eosinol (with medicine dropper) and agitate the slide. Generally one or two applications are sufficient to remove the gentian violet and at the same time counterstain the section red.
11. Treat with carbolxylene for thirty seconds.
12. Treat with two changes of pure xylene, fifteen seconds each.
13. Mount in gum dammar.

Results: Gram-positive organisms (bacteria and fungi) are stained violet; gram-negative organisms are unstained; the background is bright red.

For emergency examination, fresh tissues are fixed as follows:

A 10 per cent formaldehyde solution is brought to a boil, and thin blocks of the tissue are dropped into it. The container is placed in a paraffin oven at 56° to 60° C. for fifteen minutes' fixation. Then frozen sections are prepared and stained as already described.

Preparation of Eosinol.—Dissolve 5 Gm. of aqueous eosin in 10 c.c. of distilled water. Precipitate it by adding 10 c.c. of glacial acetic acid and 2 c.c. of concentrated hydrochloric acid, and mix with a glass rod.

Incubate the resulting coagulum at 56° C. for from twelve to sixteen hours, or until all the water has evaporated.

Dissolve this dehydrated acid eosin in 10 c.c. of absolute alcohol or anhydrous isopropanol and 20 c.c. of acetone, stirring with a glass rod for several minutes and placing it in a paraffin oven for two hours. Let the undissolved portion, which is to be discarded, settle to the bottom of the container. Remove the clear portion with a clean, dry pipette, add it to 1,500 c.c. of carbolxylene (1 part of pure phenol crystals in 3 parts of neutral xylene). Some precipitate will form, which will settle to the bottom of the container. The clear portion is eosinol. The solution keeps indefinitely.

Owing to the variation in the staining power of various brands of powdered eosin, it may be necessary to standardize the solution by staining control sections and adjusting the strength of the eosinol by reducing or increasing the amount of carbolxylene.

REVIEWS

Books and Monographs for Review should be sent direct to the Editor,
Dr. Warren T. Vaughan, Professional Building, Richmond, Va.

Poisons, Their Isolation and Identification*

AS NOTED in the Foreword, the number of poisoning cases in Egypt is sufficiently large to engage permanently a force of chemists in the necessary medicolegal determinations. This book, written by the late Director of the Medicolegal Laboratory of the Ministry of Justice is, therefore, based upon an extensive and comprehensive experience.

Each procedure detailed has been personally used and found reliable by the author. The book is hence a record of practical experience and as such should prove of great value to others engaged in this field.

The author presents in Chapter X a systematic scheme for the identification of alkaloids embodying much that is original. A complete author index and a comprehensive general index make the contents of the book readily available.

Intended as a practical manual for all chemists who have to deal with poisoning cases, this book should fill a definite need.

The Compleat Pediatrician†

THE necessity for a third edition in six years is ample testimony to the practical clinical utility of this book. Comprehensive and well organized, it embodies perhaps the most complete epitome in small compass of pediatrics yet available.

It can be consulted with confidence that the information sought will be found and that when found it will be in readily applicable form.

This new and revised edition should prove as welcome and as useful to the clinician as did its predecessors.

The Essentials of Applied Medical Laboratory Technic‡

A PROFUSELY illustrated volume describing the generally accepted routines in clinical laboratory work, this book should be of especial value for the clinical pathologist's assistant and can be very highly recommended to all technicians. Indeed, it will probably become a widely used textbook for technicians.

Embalming Fluids§

A COMPREHENSIVE discussion of the art of embalming from the earliest to the present time, this book contains much information of interest, not only to embalmers, but also to physicians and pathologists and all who are interested in the historical aspects of this subject.

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*Poisons, Their Isolation and Identification. By Frank Bamford, B.S., late Director of Medicolegal Laboratory, Ministry of Justice, Cairo, Egypt. With Foreword by Sydney Smith, M.D., F.R.C.P. Washable binding, 344 pages, 21 illustrations, $4.00. P. Blakiston's Son & Co., Philadelphia, Pa.

†The Compleat Pediatrician. Practical, Diagnostic, Therapeutic, and Preventive Pediatrics. By W. C. Davison, M.D., Professor of Pediatrics, Duke University School of Medicine. Cloth, ed. 3, 256 pages, $4.00. Duke University Press, Durham, N. C.

‡The Essentials of Applied Medical Laboratory Technic. Details of How to Build and Conduct a Laboratory in Hospital or Office at Small Cost. By J. M. Feder, M.D., Director of Laboratories and Allergic Service, Anderson County Hospital, Anderson, S. C., Cloth, 234 pages, profusely illustrated, two plates in color, $5.00. Charlotte Medical Press, Charlotte, N. C., 1940.

§Embalming Fluids. Their Historical Development and Formulation From the Standpoint of the Chemical Aspects of the Scientific Art of Preserving Human Remains. By Simon Mendelsohn, F.A.I.C., Consulting Chemist. Cloth, 166 pages, 6 figures and frontispiece, $4.00. The Chemical Publishing Co., New York, N. Y.

Histopathology of the Peripheral and Central Nervous Systems*

HERE is a volume that should always be available as a companion piece to any text on clinical neurology. Its function is similar to that of the texts of general pathology in relation to texts on general medicine. It is very complete, with discussion of very recent studies, including the many forms of meningitis and encephalitis and the myopathies.

The book is abundantly and well illustrated.

Laboratory Text in Pharmacology†

THIS excellent manual of experimental exercises is intended to serve as a supplement to the more complete texts. It was developed over the course of several years; hence it has been subjected to the vicissitudes of actual laboratory use.

Valuable tips are given as to the proper technique of administering drugs to various mammals. The reviewer can recall a ruined experiment which would have had better results had he been forewarned "that rabbits are very adept at kicking syringes and bottles off the table."

The second portion of the book deals with prescription writing and materia medica, the former of which gives evidence of becoming a lost art under the pressure of commercial preparations. Throughout, frequent references are given to the current literature.

The manual can be highly recommended.

Pathological Histology‡

THE student of pathology and the physician looking for a "refresher" course will find this a text of outstanding value for the purpose. The pathologist, too, will find it a valuable addition to his reference library.

The text is succinct and authoritative. The numerous illustrations—Finlay process transparencies of excellent quality excellently reproduced—are well chosen and worthy of the highest commendation. They are, indeed, an outstanding feature of the book which can be highly recommended without reserve. Altogether an excellent "buy," it is moderately priced and well worth its cost.

Erratum

On page 730 of the January number of the JOURNAL in the article by Ida Kraus, Ph.D., and Sol Dulkin, B.S., entitled "The Determination of Hippuric Acid in Urine," the eleventh line of the paragraph below Table I should read "ten normal sulfuric acid" instead of "tenth normal sulfuric acid." On page 731 the seventh line of the second paragraph below Table II should read "10 normal sulfuric acid" instead of "1:1 sulfuric acid."

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*Histopathology of the Peripheral and Central Nervous Systems. By George B. Hassin, M.D., Professor of Neurology, University of Illinois, College of Medicine, Attending Neurologist, Cook County Hospital, Chicago. Cloth, ed. 2, (revised and enlarged), 302 illustrations, 554 pages, $7.50. Paul B. Hoeber, Inc., Medical Book Department of Harper & Brothers, New York, 1940.

†Laboratory Text in Pharmacology. By Robert P. Walton, Professor of Pharmacology, School of Medicine, University of Mississippi. Paper, 85 pages, $1.50. J. B. Lippincott Company, Philadelphia, London, Montreal.

‡Pathological Histology. By Robertson F. Olgivie, M.D., F.R.C.P. (Edin.), Lecturer in Pathology, University of Edinburgh; Senior Pathologist, Royal Infirmary, Edinburgh; Pathologist, Deaconess Hospital, Edinburgh; Examiner in Pathology for the Triple Qualification. Foreword by A. Murray Drennan, M.D., F.R.C.P. (Edin.), Professor of Pathology, University of Edinburgh. Cloth, 332 pages, 220 colored plates, $8.50. A William Wood Book. Williams & Wilkins Co., Baltimore, Md.